Medical Genetics

Volume I
Basic Genetics

Part V
Pathogenetics

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Spectrum of Medical Genetics

Basic Genetics	Clinical Genetics
Part I: Molecular Genetics Part II: Biochemical Genetics Part III: Physiological Genetics Part IIII: Cytogenetics Part V: Pathogenetics Part VI: Pharmacogenetics Part VII: Oncogenetics Part VIII: Immunogenetics Part IX: Formal Genetics Part X: Population genetics Part XI: Developmental Genetics Part XII: Genomics Part XIII: Transcriptomics Part XIV: Proteomics	Part I: Chromosomal Aberrations Part II: Congenital Malformations Part III: Inborn Errors of Metabolism Part IV: Mitochondrial Disorders Part V: Genetic Systemic Syndrome Part VI: Genetic Diseases of The Nervous system Part VII: Genetic Diseases of The Endocrinal system Part VIII: Genetic Diseases of The Cardio-Vascular system Part IX: Genetic Diseases of The Respiratory system Part X: Genetic Diseases of The Gastro-Intestinal system Part XI: Genetic Diseases of The Urinary system Part XII: Genetic Diseases of The Muscular system Part XIII: Genetic Diseases of The Skeletal system Part XIV: Genetic Diseases of The Blood system Part XV: Genetic Diseases of The Immunity system Part XVI: Genetic Diseases of The Male Genital system Part XVII: Genetic Diseases of The Female Genital system Part XVIII: Genetic Diseases of The Ocular system Part XIX: Genetic Diseases of The Auditory system Part XX: Genetic Diseases of The Skin Part XXI: Genetic Psychiatric Disorders
Diagnostic Genetics	**Therapeutic Genetics**
Part I: Molecular Diagnostic Techniques Part II: Cytogenetic Diagnostic Techniques Part III: Biochemical Diagnostic techniques Part IV: Prenatal Diagnosis Part V: Pre-Implantation Diagnosis Part VI: Per-Symptomatic Diagnosis Part VII: Conventional Diagnostic Techniques	Part I: Pharmacologic Therapy Part II: Nutritional Therapy Part III: Replacement Therapy Part IV: Transplantation Therapy Part V: Stem Cell Therapy Part VI: Surgical Intervention Part VII: Genetic Therapy Part VIII: Fetal Therapy Part IX: Conventional Therapy
Prophylactic Genetics	**Applied Genetics**
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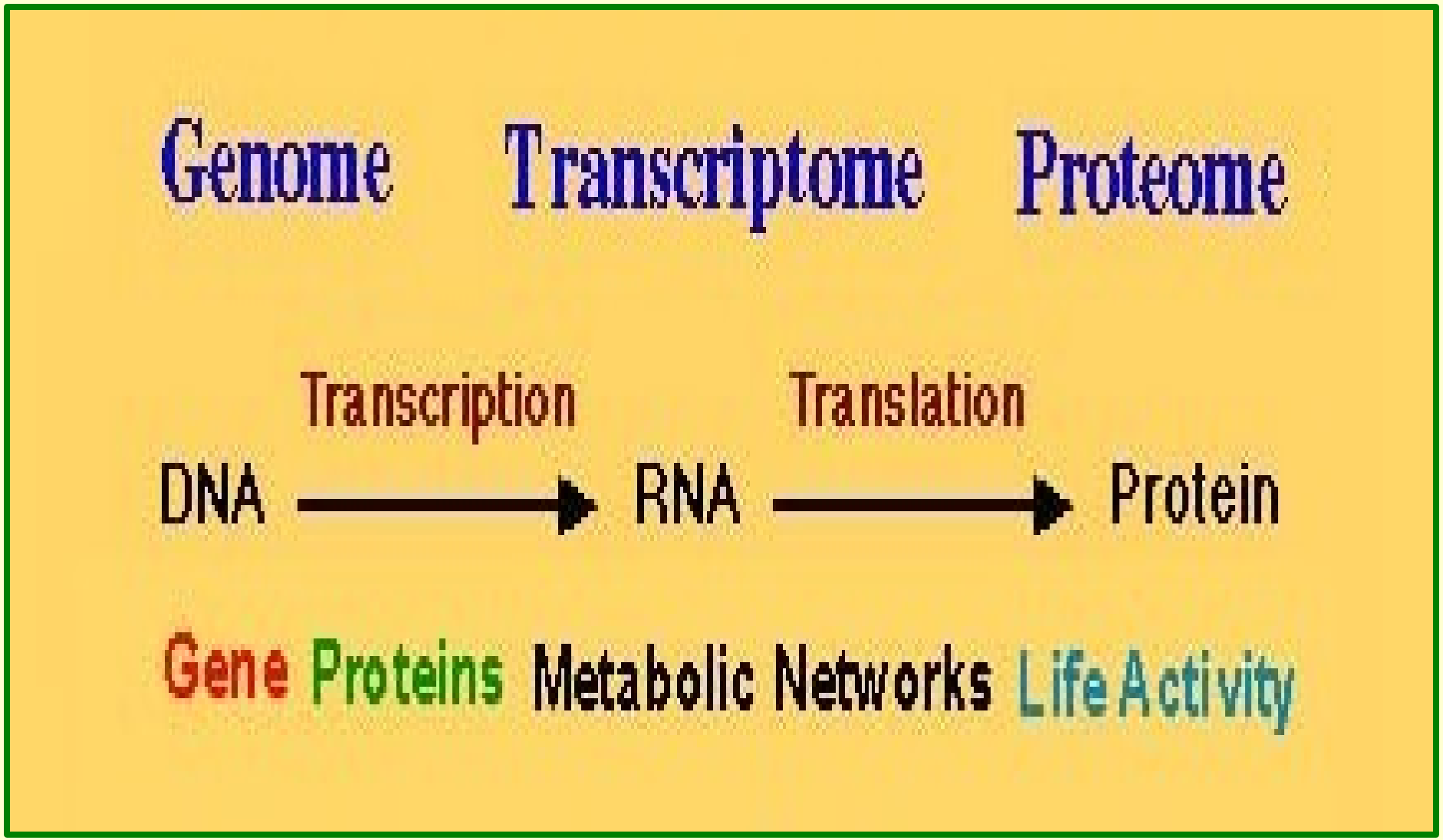
Dogma of Molecular Biology

Relation Between The genetic Material and Life Activities

Life Activities at The Molecular level



Dogma Of Molecular Pathology In Health And Disease

Genome → Transcriptome → Proteome

Transcriprion — Translation

DNA → mRNA → Protein

Gene — Protein — Metabolic Network — Cellular Function — Life Activity

Mutant Gene → Abnormal mRNA

↓

Deficient/Defective/Excess Product

Protein (Structural/Catalytic) – RNA

↓

Disturbed Metabolic Networks → Disturbed Cell Function

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Deranged Physiological Activities → Disease

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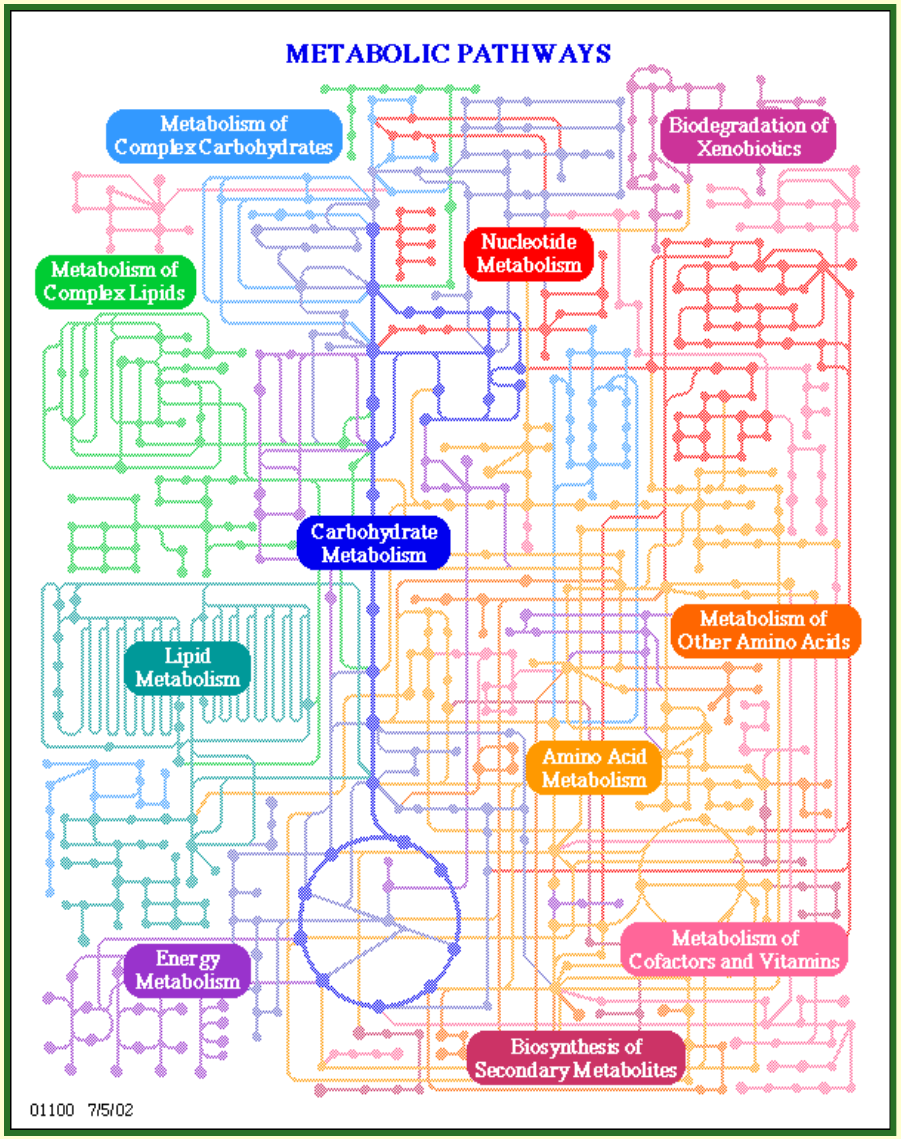
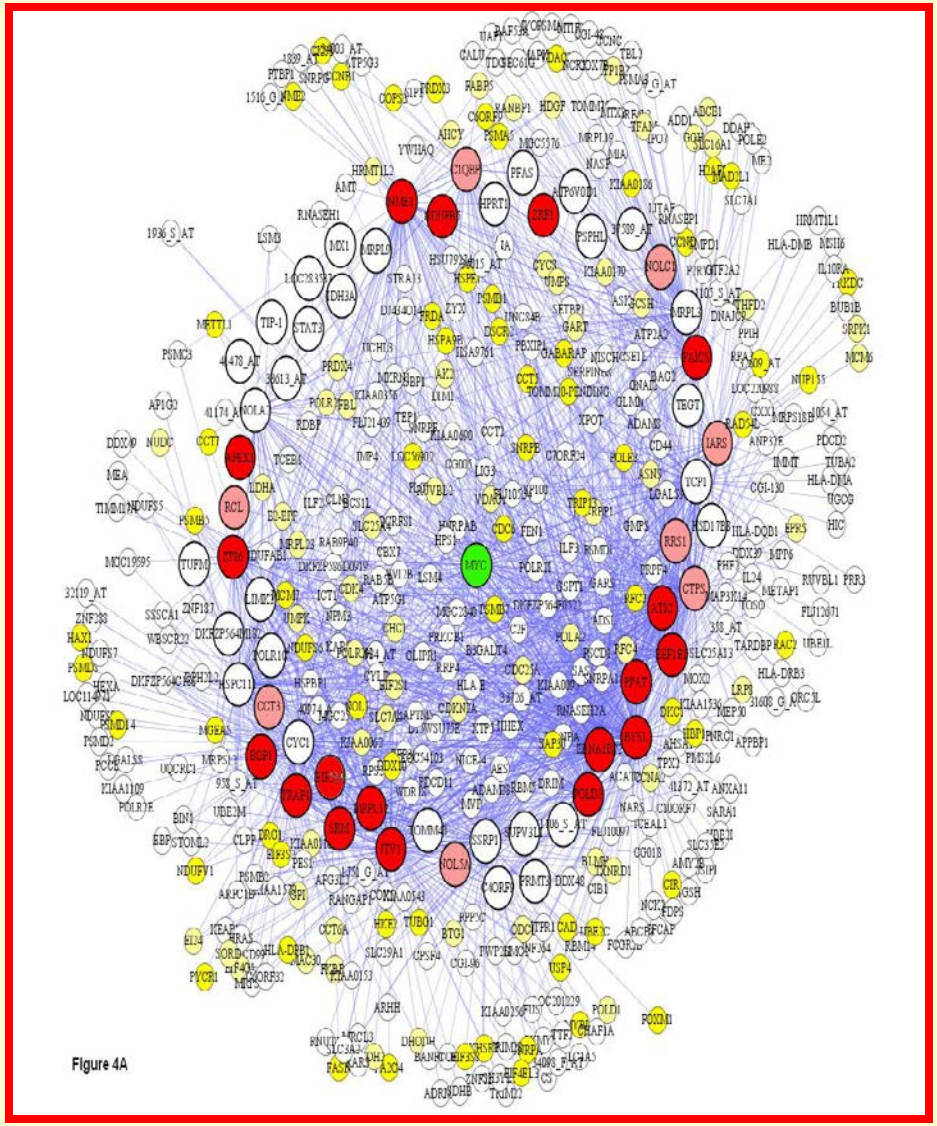
Genetic Disorder – Immunodeficiency – Congenital Anomaly – Cancer

The Concept Of Metabolic Networks





Pathogenesis Of Genetic Diseases

Genetic diseases result, primarily, from defect(s) or change(s) in structure of genes. This change in gene structure, termed mutation, in most instances results in disordered gene function leading to defective or deficient production of the gene product, or the protein.

The resulting defective or deficient protein leads to limited or widespread disorder(s) in one or more metabolic networks involving the defective protein in its pathway, thus ending ultimately in, and leading to, pathophysiological alterations and development of disease.

Mutation

Mutation is defined as any alteration in the structure of the genetic material at any of its organizational levels. These levels comprise : single nucleotides, DNA, RNA, genes, chromosomes, mitochondrial DNA (mtDNA), up to the whole genome.

The effects of mutations differ widely according to many factors. These factors include the nature and target of the mutagen, the timing and magnitude of the resulting damage, and the balance between synergistic effects and antimutation mechanisms of the genetic material.

Mutations may occur without an identifiable cause and are termed spontaneous mutations, or they may occur secondary to exposure to a known cause, and are referred to as induced mutations. Factors that can induce mutations in the genetic material are called mutagens.

MUTAGENS

Mutagens are factors that can cause mutations. With the exception of energy-induced changes in the stability of the bonds of the nucleotides that induce spontaneous mutations, mutagens are environmental agents potentially capable of altering or damaging the genetic material upon exposure to their effects.

The damaging effects of mutagens depend on many factors including the nature and dose of the mutagenic agent, the target of the mutational insult and the timing of exposure to the mutagenic effects in relation to specific ongoing cellular processes, like cell division, differentiation, migration and protein synthesis.

According to their nature, mutagens are classified into three main categories:

1. Chemical mutagens
2. Physical mutagens
3. Biological mutagens.

Chemical mutagens comprise countless numbers of chemical compounds like simple and complex organic compounds, insecticides, asbestos, herbicides, heavy metals, etc.
Physical mutagens include particulate radiations like alpha and beta particles, solar radiation, UV waves, thermal and mechanical agitation of nucleic acids, etc.
Biological mutagens include living microorganisms like many types of viruses (rubella, cytomegalovirus, herpes virus), and Toxoplasma.

According to their effects, mutagens are classified into four main categories:

1. **Carcinogens**
2. **Clastogens**
3. **Teratogens**
4. **Non-specific Mutagens.**

Carcinogens are mutagenic agents capable of inducing malignant transformations in affected cells.

Clastogens are mutagenic agents that cause chromosome breaks in affected cells.

Teratogens are mutagenic agents that induce development of malformations in embryos and fetuses exposed to their effects.

Non-specific mutagens are mutagens that cause non-specific deleterious effects of the genetic material.

Mutagens : types, effects and exa

Mutagens	Effects	Examples
Carcinogens	Carcinogenesis And Tumor Formation.	Chemical : Aflatoxins Biological : Retroviruses Physical : X-ray Irradiation
Clastogens	Chromosome Breaks, Deletions, Rearrangements.	Chemical : Bleomycin Biological : HIV virus Physical : X-ray Irradiation
Teratogens	Congenital Malformations.	Chemical : Valproate Biological : Toxoplasma G Physical : X-ray Irradiation
Non-specific Mutagens	Non-specific Damage To The Genetic Material.	Chemical : Innumerable Physical : X-ray Irradiation Biological : Toxoplasma, Viruses

TYPES OF MUTATION

1. Spontaneous versus Induced
2. Somatic versus Germinal
3. Nuclear versus Mitochondrial
4. Static versus Dynamic
5. Persistent versus Reversible
6. Point . Genic . Chromosomal . Genomic
7. Base . Sugar . Phosphate group.
8. Pathological versus Non-Pathological

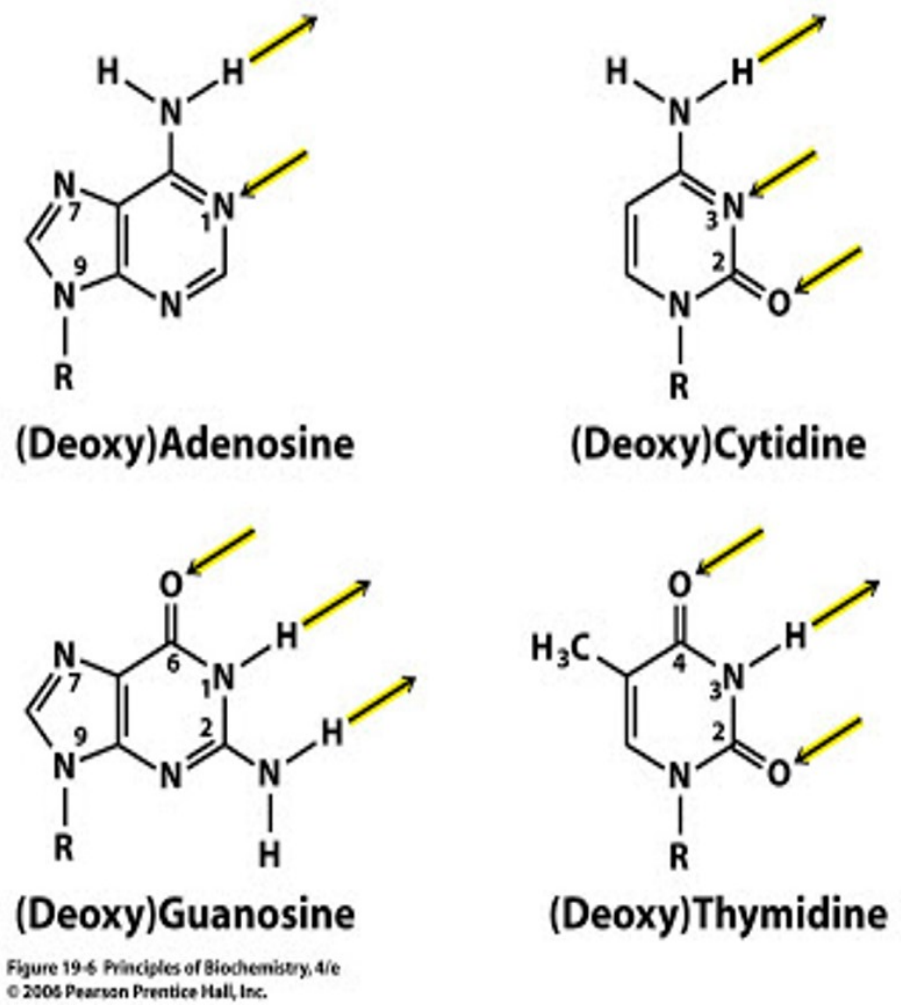
Spontaneous versus Induced mutation

Spontaneous mutations refer to mutations that occur without known mutagenic effect. In fact, nothing happens in the genome spontaneously, rather the spontaneous invisible and undetectable changes in the energy states of the electrons of the bonds of the nitrogen bases of the nucleotides, which are normal events in the atoms, result in changes in the direction and stability of these bonds, e.g., tautomeric shift. If conditions favoring the persistence of these bond changes for enough time are present, then change of one adenine base to another adenine base, or change of one pyrimidine base to another pyrimidine base via transition might occur and persist and a, seemingly, spontaneous mutation results.

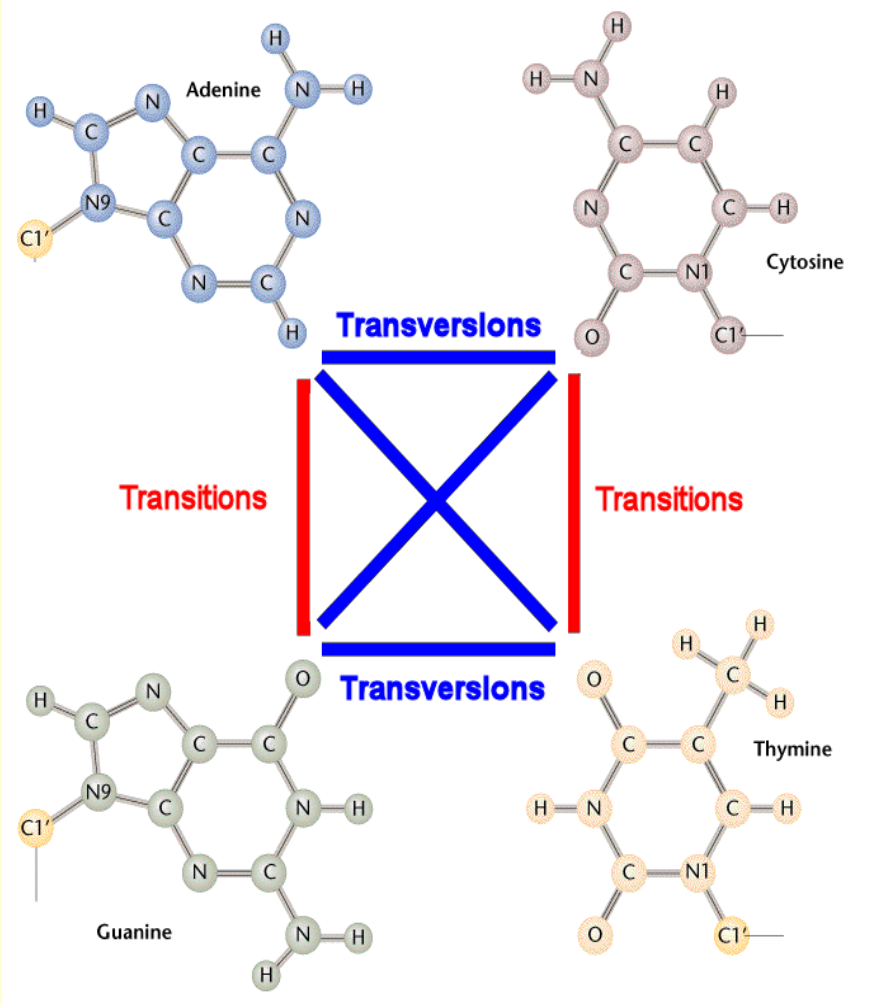
The same can also result from transversion where an adenine base gets bound to a pyrimidine base or vice versa.

Molecular Mechanisms of Spontaneous Mutations

Base Tautomerism



Base substitution



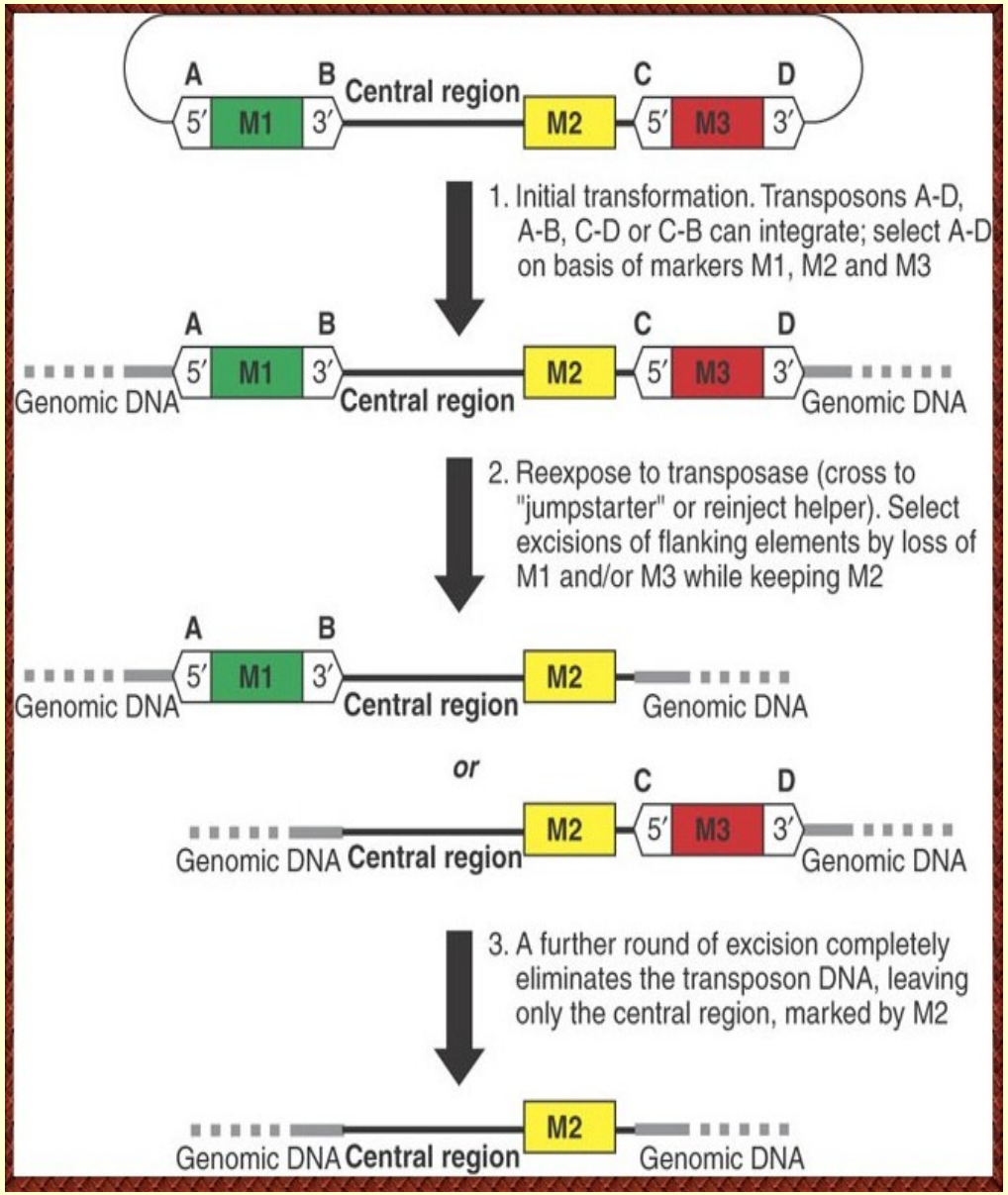
It must be kept in mind that current traditional definitions of spontaneous mutations are not totally accurate and are in need of revision. For example, mutations that happen during DNA replication are considered as spontaneous mutations, whereas, actually, they are induced mutations due to failure of the DNA proofreading system which might be secondary to defective DNA repair and surveillance mechanisms. The spectrum of conditions that favor the triggering of spontaneous mutations of the genetic material, and allow the persistence of these mutations long enough to be fixed and become permanent changes in the genome is still vague. Some of these factors include the structural variant of DNA (Z versus β form), the specific arrangement of the various segments of the gene, the size of the gene, the relative abundance of mutable sites or hot spots in the gene, the gene locus and the local cellular metabolic situations like calcium concentration and acidity of the cytoplasm and nucleoplasm.

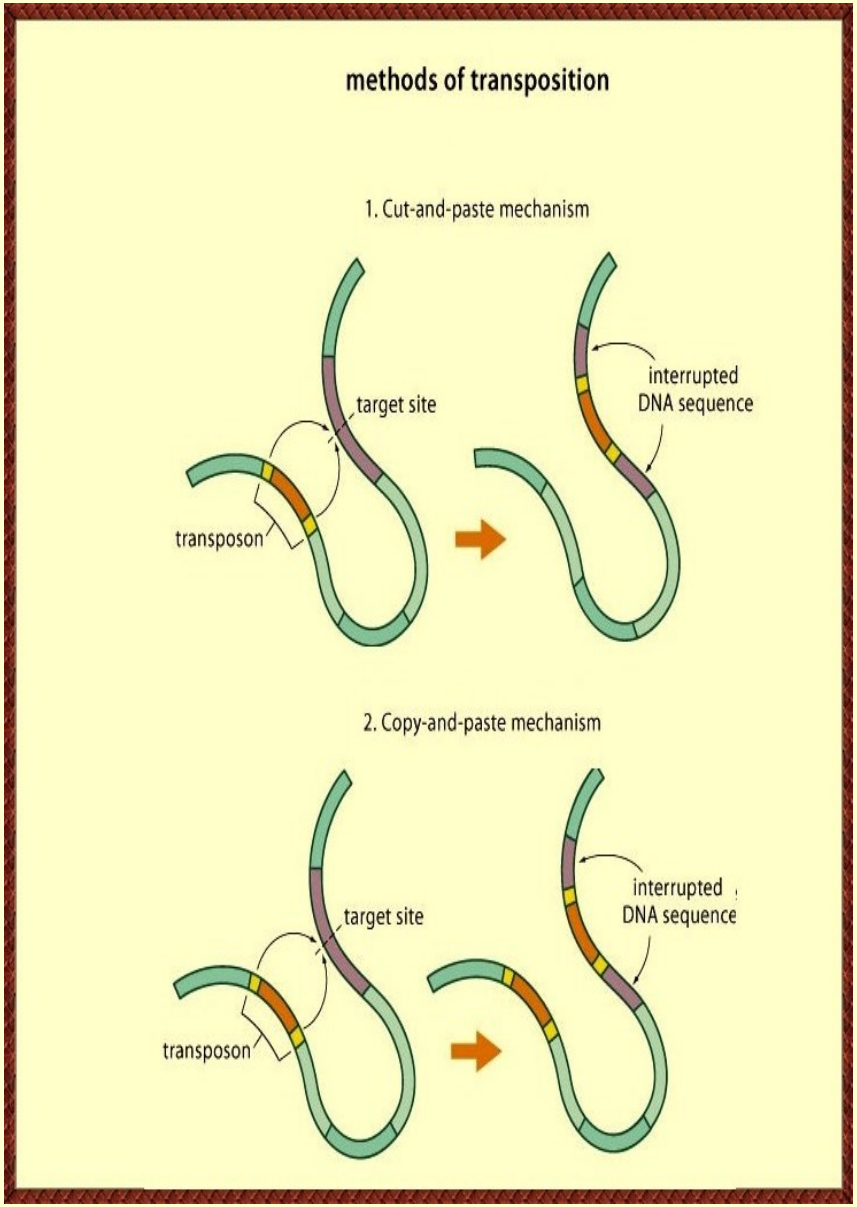
Transposons and Transposons-Induced Mutations

Transposons are sequences of nuclear DNA that can move or transpose themselves to new positions within the nuclear genome of the cell. The mechanism of transposition can be either by synthesis of a copy of the transposon segment and inserting the new copy into another site of the genome (Class I Retrotransposons) or through separation of the transposon sequence itself from its site and its insertion into a new site (Class II DNA transposons).

Although transposons and related transposon-like repetitive elements constitute a large proportion, nearly 44%, of the human genome, only a small proportion (<0.05%) of these elements are still active. Recent evidence indicates that 35–40 subfamilies of repetitive elements (*Alu*, L1 & SVA elements and possibly HERV-K elements) remain actively mobile in the human genome.

Methods & Mechanisms Of Transposition





According to the extent and magnitude of the mutation-induced damage of the genetic material, mutations might be classified into four major categories :

1. Point mutations
2. Small mutations
3. Gross mutations
4. Genomic mutations.

Point mutations involve deletion, addition or change of one single base, or nucleotide, of the gene.

Small mutations involve deletion, addition or change of two or more bases up to large segments of the gene.

Gross mutations involve large changes comprising deletions, duplications, inactivation and rearrangements of one or more genes or one or more chromosomes.

Genomic mutations affect the whole nuclear genome either via inactivation (imprinting) or reduplication.

Quantitative Classification of Mutation

According to the target of mutation, four major types of mutation can be delineated:

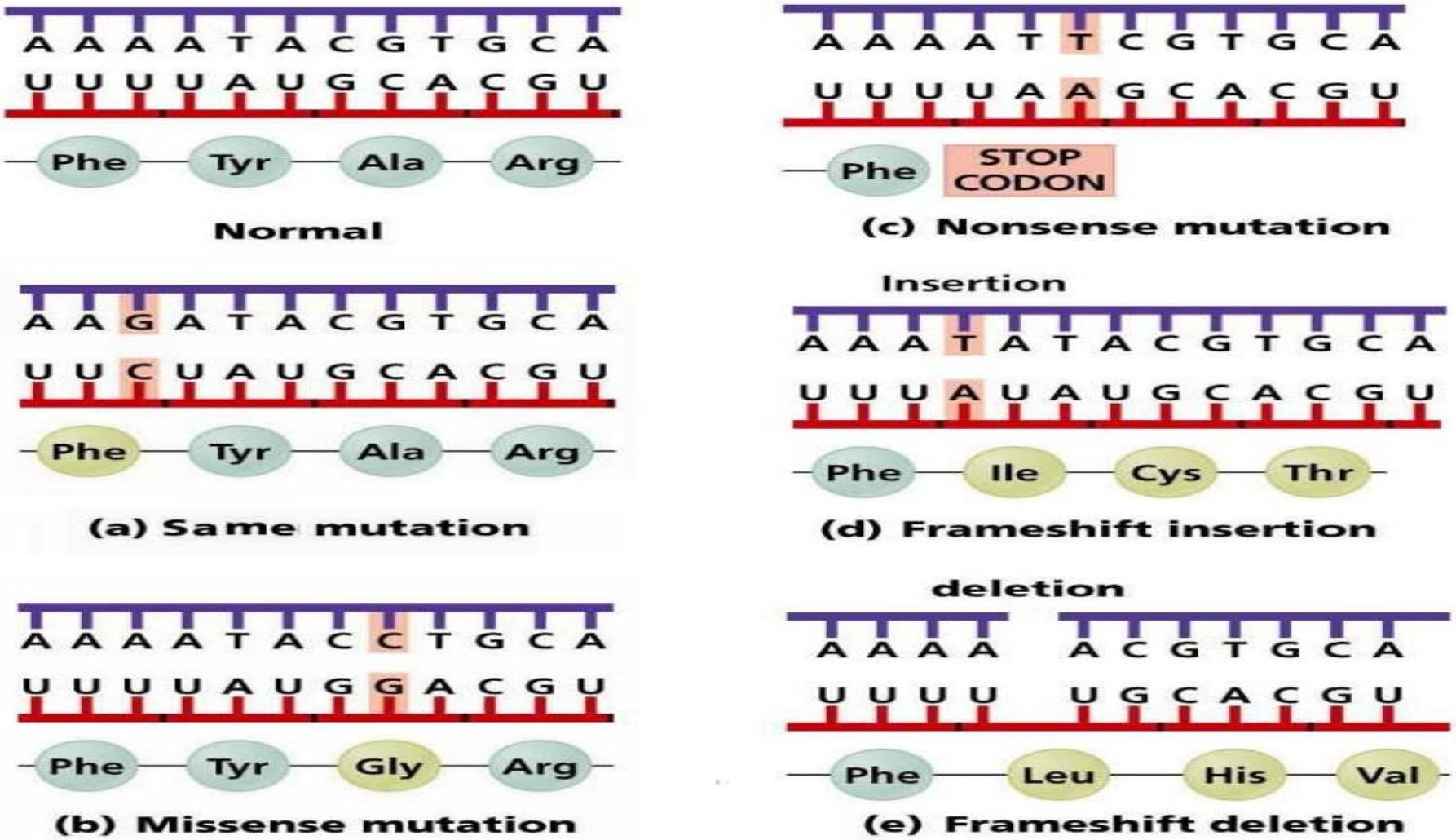
1. Point mutation caused by change of one single base. It comprises five different types: missense, same sense, non-sense, re-sense and single base frame shift mutation.

2. Small mutation involving more than one base up to parts of genes or few genes.

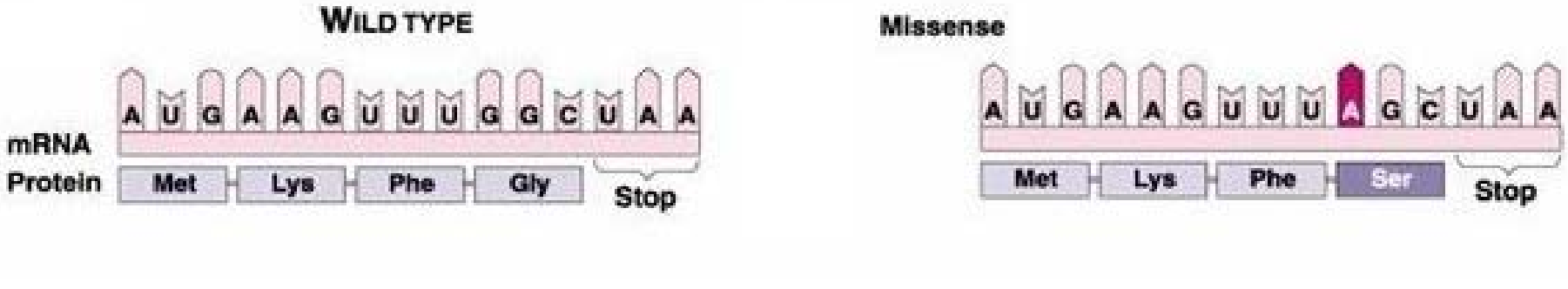
3. Gross mutation comprising chromosomal aberrations.

4. Genomic mutation involving the whole genome, either the haploid (23) germ cell genome or the diploid (46) somatic genome (triploidy, tetraploidy, genomic imprinting).

1. Types of Point Mutations



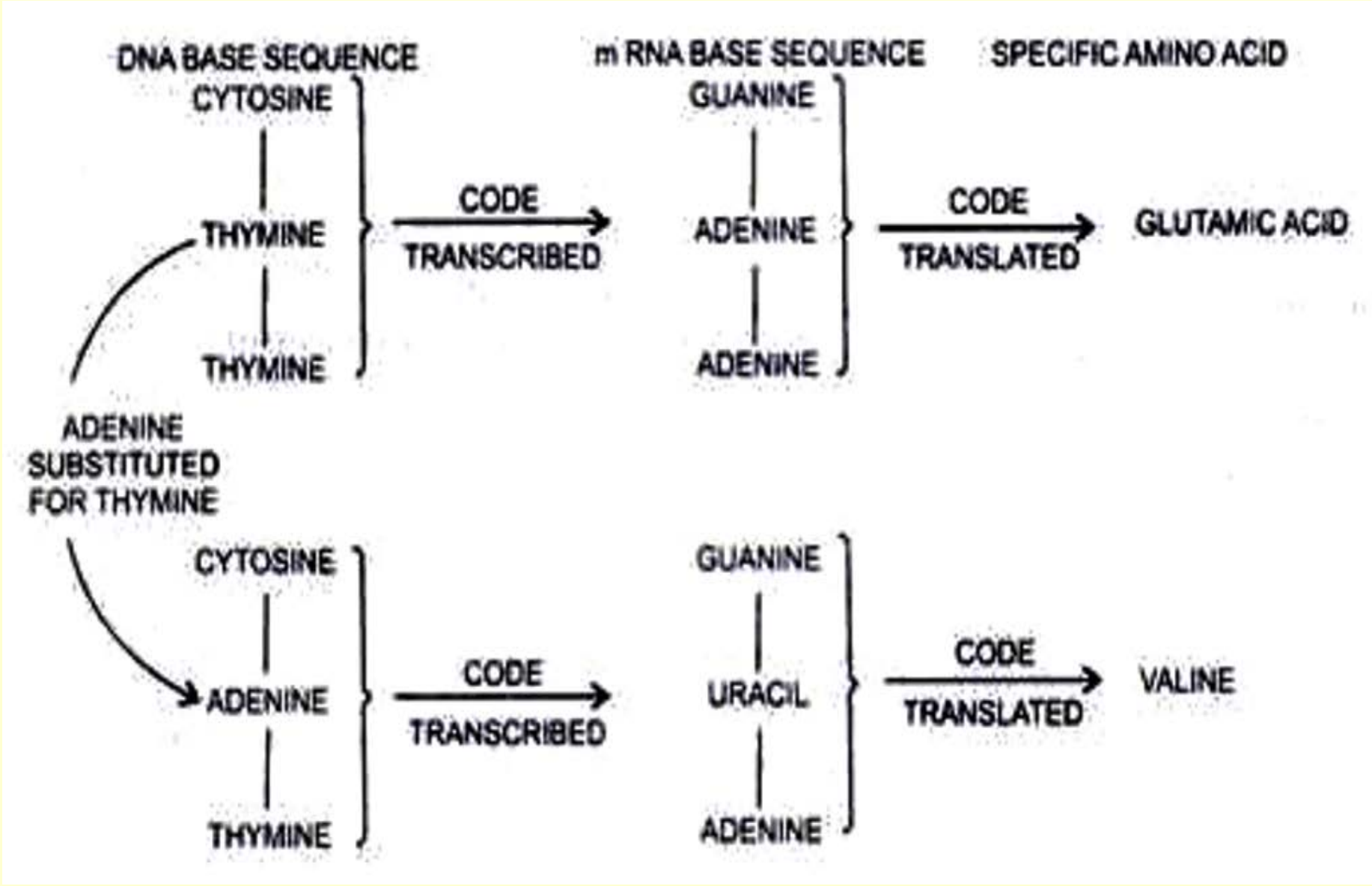
1. Mis-sense mutation



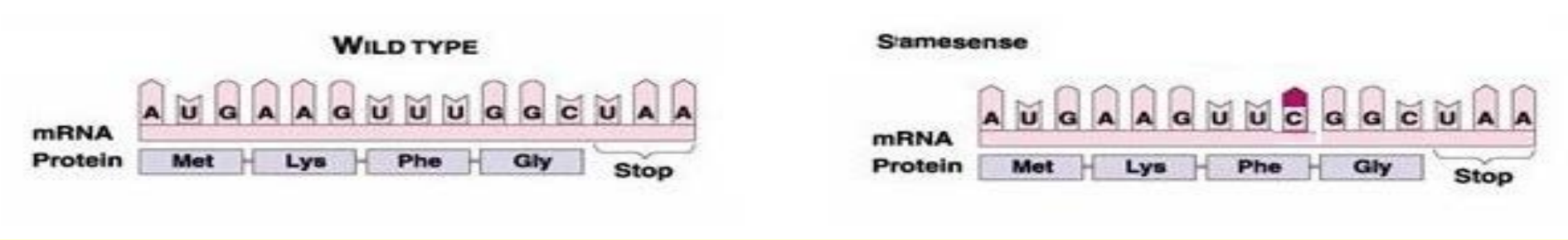
In this type of point mutation, change of one nucleotide of a codon specifying a specific amino acid by another nucleotide might change the codon to a different codon specifying a different amino acid. For example, change of (GGC) coding Glycine to (AGC) coding Serine.

If the wild type amino acid mediates a particular function in the protein that can not be performed by the new amino acid, then defect(s) in protein function(s) might result leading, in most instances, to altered pathophysiological effect(s) and disease according to the altered function of the new protein.

Missense Point mutation of Sickle cell anemia



2. Same-sense mutation



In this type of point mutation, replacement of one nucleotide of a codon specifying a particular amino acid by another nucleotide, turning the codon into another codon specifying the same amino acid, due to degeneracy of the genetic code, occurs. For instance, change of (UUU) coding Phenylalanine to (UUC) which, also, codes Phenylalanine.

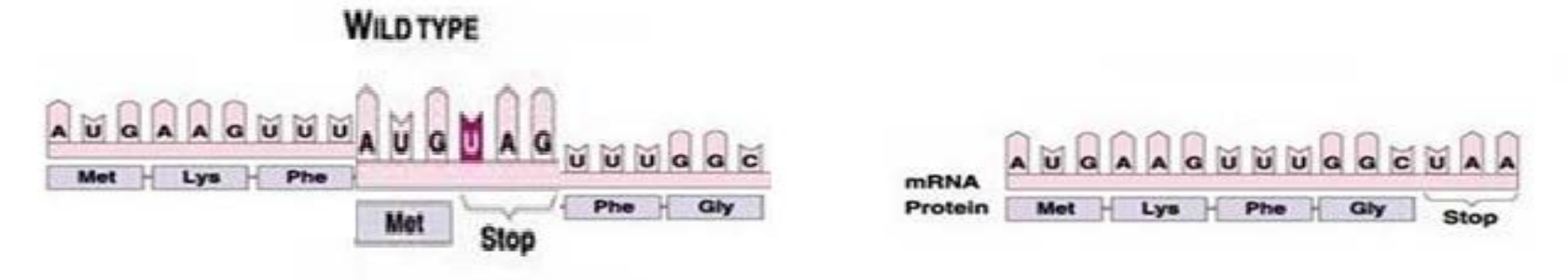
In this type of point mutation, no change(s) in the protein function(s) occur since the protein has the same native structure, and no pathophysiological alterations are noted and no disease(s) develops.

3. Non-sense mutation

This type of point mutation entails change of one nucleotide of a functional codon by another nucleotide turning the codon into a stop codon. For example, change of (AAG) coding Lysine to (UAG) which is a Termination or Stop codon that do not code any amino acids.

These mutations cause cessation of translation, truncation or premature termination of protein synthesis and production of shorter incomplete proteins. If the missing part of the protein is functionally important, these mutations result in functional deficiency, pathophysiological alterations and development of disease state.

4. Re-sense mutation



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These mutations cause cessation of translation, truncation or premature termination of protein synthesis and production of shorter incomplete proteins. If the missing part of the protein is functionally important, these mutations result in functional deficiency, pathophysiological alterations and development of disease state.

5- Frameshift mutation

Frameshift point mutations involve insertion or deletion of one nucleotide from a specific codon leading to shift of the reading frame of the mRNA and change of the amino acid sequence of the protein, starting from the mutated codon till the end of the protein.

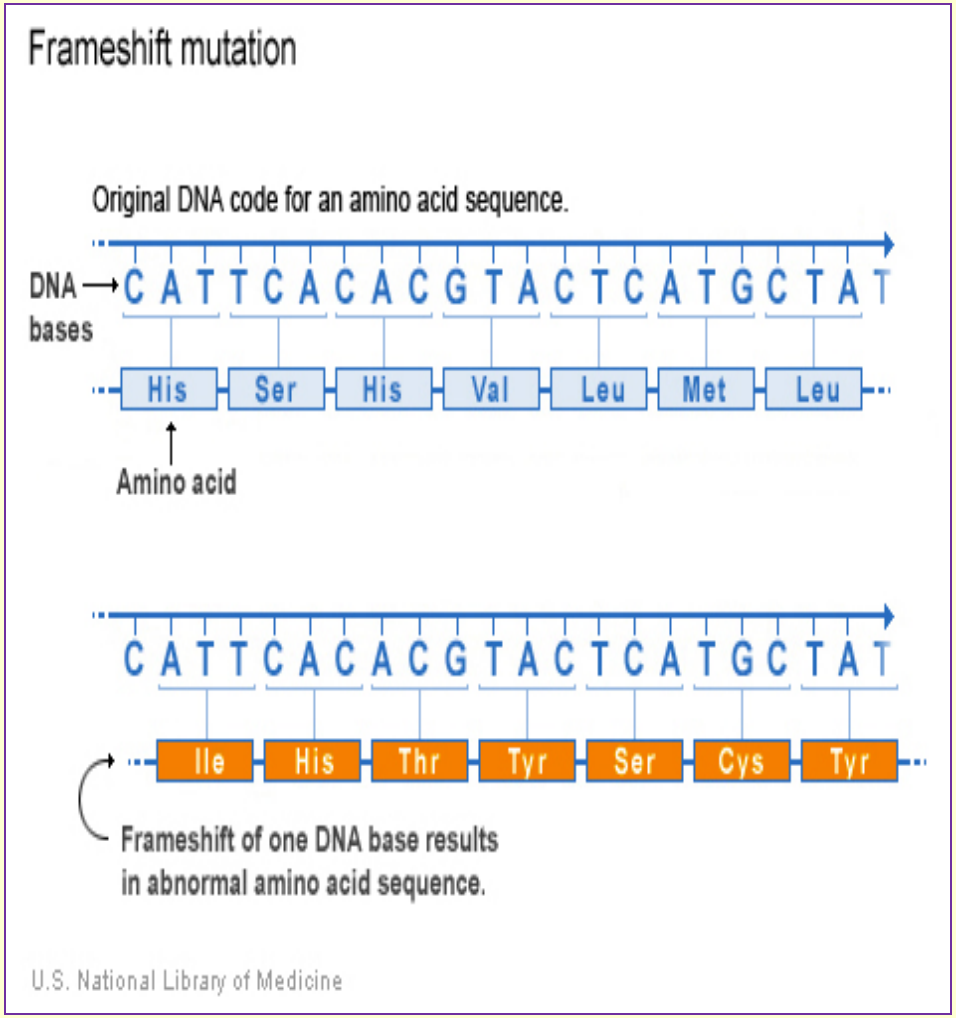
If 3 bases in sequence (one codon) are removed or inserted, then a deficiency, or addition, of one amino acid in the final protein product results, respectively.

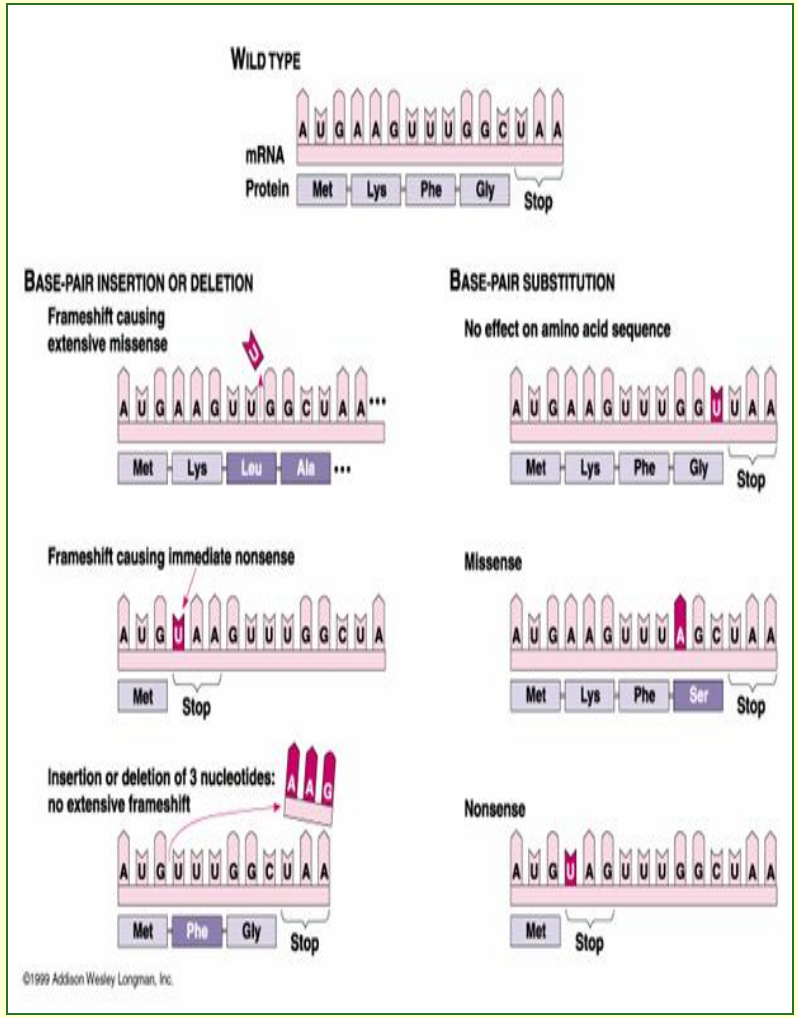
The effects of this type of mutation depends largely on the specific structural - functional relationship of the protein domains. If the missing amino acid is critical for protein function, pathophysiological alterations result and disease consequences might happen.

The same effects might occur if the extra amino acid affects the stability of the protein or its spatial configuration, thus leading to enhanced degradation of the protein and disease manifestaions might occur.

Frameshift mutations might also result in creation of a stop or termination codon instead of a functional codon along the reading frame of the gene and the mRNA. In these situations, truncation of the resulting protein, or synthesis of a shorter polypeptide chain happen. The resulting pathophysiological alterations in these types of mutations depend on the role(s) played by the missing or deficient part of the protein. If they constitute integral functional domains of the protein, disease consequences result, otherwise the resulting effects might be limited to changes in the physico-chemical properties of the protein, eg change in the molecular weight or in the electro-phoretic behavior of the protein.

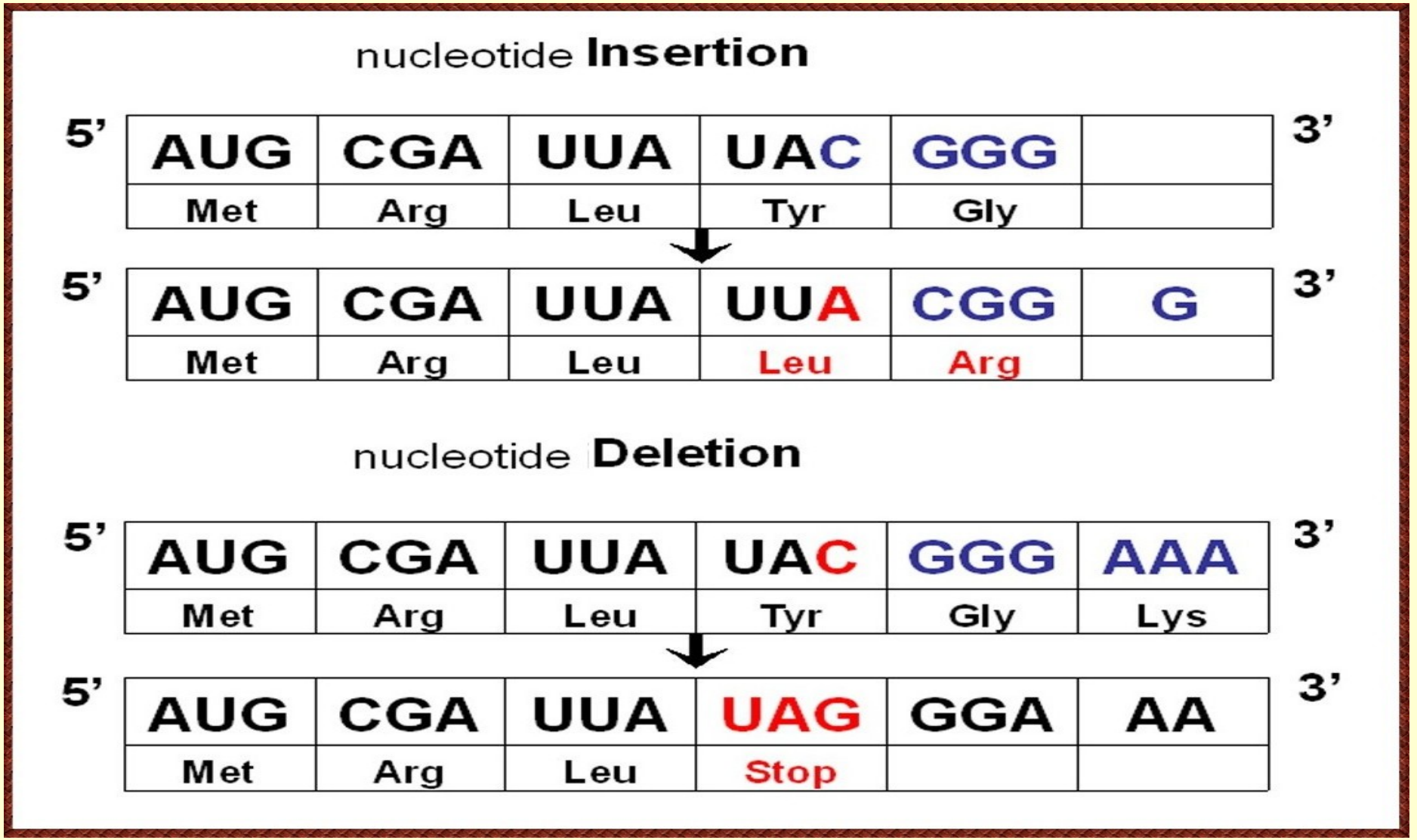
FRAMESHIFT MUTATIONS



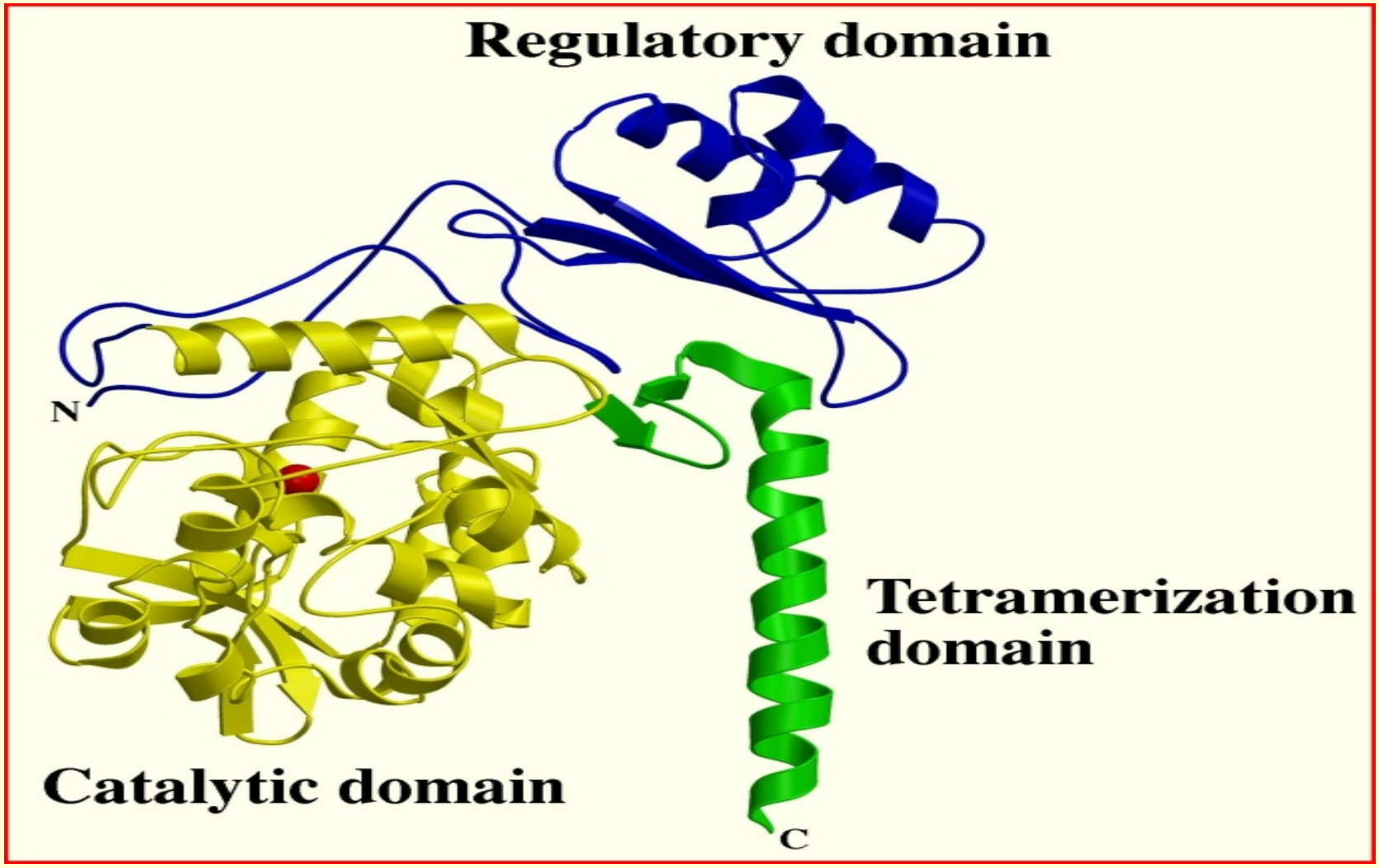


FRAMESHIFT MUTATIONS
Insertion, Deletion Of Amino Acids

Frameshift Mutation
Creation of termination codons



The Concept of Protein Domains



The Concept Of Protein Domains And Its Relation To The Effects Of Mutation

The amino acids of proteins are structurally and functionally organized into distinctive domains. Each domain consists of a defined number of specific amino acids, and performs a particular role either in shaping and maintaining the structural configuration of the protein (structural domain), or in mediating one or more of the biological function(s) of the protein (functional domain).

Each protein has its own specific domain configuration comprising the specific number and characteristic types and distribution of its domains, and because of their critical role in maintaining structural and functional integrity and identity of the protein, protein domains are markedly stable and highly conserved among most species.

Mutation results in change(s) of one or more of the protein domains depending on the site and type of the mutation, the magnitude of its effects and the nature of the protein encoded by the mutated gene.

If mutation affects one or more or all of the amino acids constituting an essential and/or an integral functional domain of the protein, a serious defect in protein function(s) results. In most instances, this pathogenetic mechanism paves the way to significant and deleterious effects on the functional integrity of the protein, leading to variable and widespread pathophysiological alterations and consequent pathogenesis of disease, secondary to loss of the role of the affected protein in the metabolic-regulatory networks and physiological pathways it mediates, or it shares in.

On the other hand, and according to the nature, the effects, and the magnitude of the causative pathogenetic mechanism, some mutations might, primarily, affect non-critical or non-integral domains of the protein leading to subtle irrelevant changes like changes in the molecular weight or in the electrophoretic mobility of the protein without any, or with inconspicuous, effects on protein functions.

In many proteins, the core functions of the integral domains depend on the presence of very few, sometimes only one single, amino acid (s) at specific critical site of the domain. If this particular single, or very few number of amino acids are changed by the mutational event, marked alterations in protein function(s) might result leading to triggering the progressive cascade of deficient / defective protein function, disturbances in metabolic-regulatory networks, progressive pathophysiological alterations, and pathogenesis of disease states.

Thus, the effects of most mutations depend largely on the resulting effects on the protein domains. This fact explains the majority of findings and observations noted in genetic diseases. For instance, it explains the marked variability of genetic mutations in causation of disease, the ability of some point mutations involving only one single base of the gene to cause serious genetic diseases, the inconspicuous pathological and clinical effects of many mutations, and the extreme wide spectrum of clinical phenotypes of genetic diseases.

2. Genic/Small Mutations

1. Loss/damage of part(s) of one or more nuclear genes (one or multiple exons/introns).
2. Loss of one or few genes.
3. Loss/damage of part(s) of mitochondrial genome
4. Loss of regulatory intergenic sequences leading to various functional defects:

   . DNA replication/repair defects

   . Splicing defects mutations

   . Telomere defects mutations

   . Transposon over activity defects.

SMALL MUTATIONS

Small mutations refer to mutations involving one region or many regions of a single gene. This definition is largely arbitrary. Thus, deletion(s) of parts of promotors, exons or introns, or of whole of these segments, of one gene are considered small gene mutations. The same, also, applies to gene rearrangements including single gene recombinational defects, gene duplication and pseudogene formation.

The effects of small genetic mutations affecting single genes depend on many factors. Regulatory gene mutations usually have profound pathological effects on functions of structural genes that are controlled by the mutated regulatory gene. The larger the number of affected genes, the more pathological alterations of physiological functions mediated by the products of these genes are to be expected.

Structural gene mutations, resulting in deficient or defective production of proteins, underlie the development of the majority of single gene disorders. Point mutations probably account for nearly seventy (70%) percent of these mutations and small mutations account for the rest of them. The effects of these structural gene mutations are, usually, limited to the consequences of the resulting defects in gene function(s) secondary to the deficient or defective gene product.

However, the need for the physiological function(s) of the deficient or defective gene product remains the most important single factors that determines the spectrum of the resulting pathogenetic effects of the mutation. Mutations of genes that code for proteins regulating cell growth and cell division have drastic effects on the cell. Ultimately, they lead to disturbed regulation of the cell cycle, enhanced apoptosis, failing cell functions and development of malignant tumors.

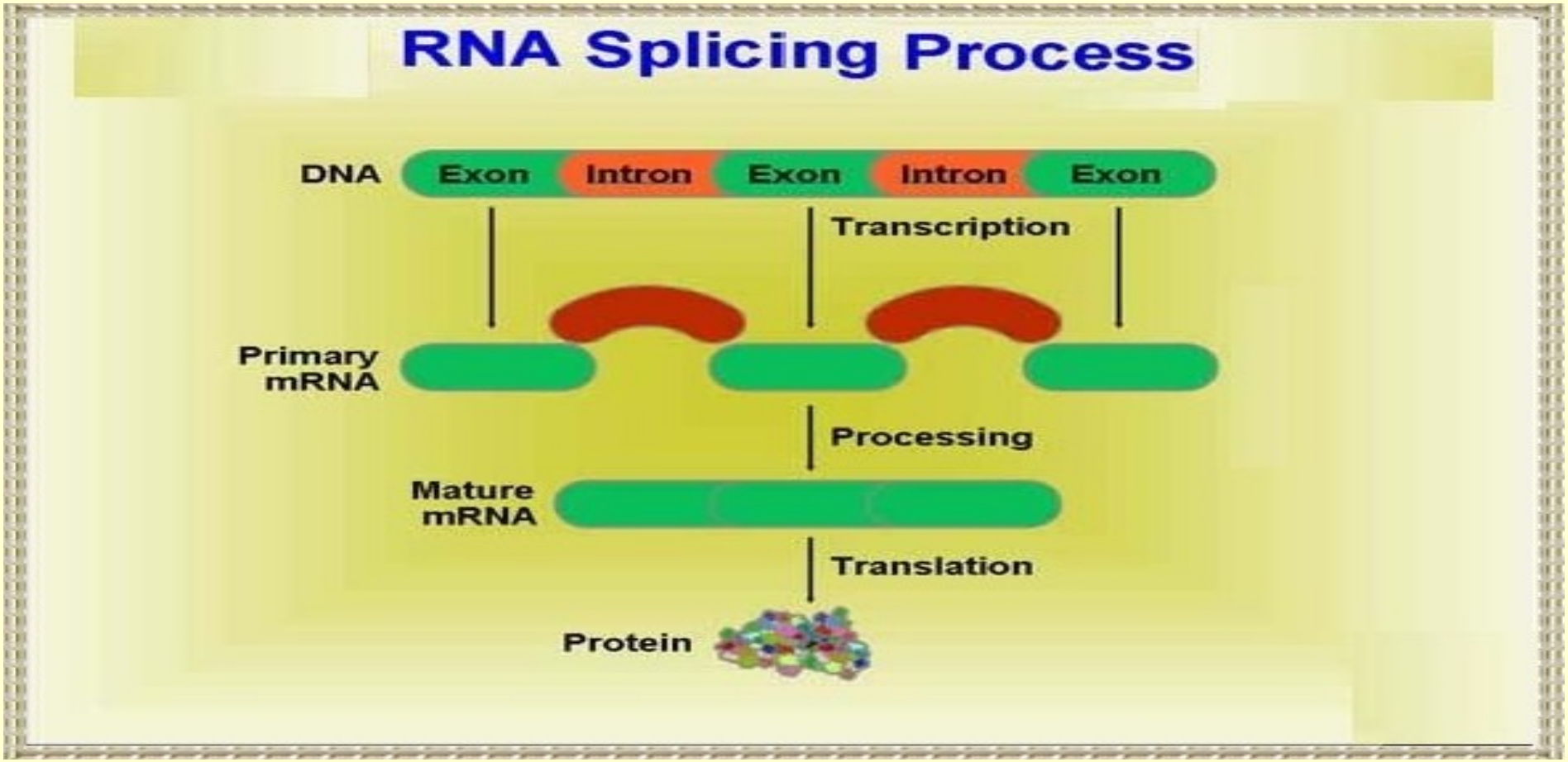
These active transposons are of great interest because they continue to produce and mediate genetic diversity, genetic heterogeneity and genetic polymorphism in human race and human populations. Similarly, much significance has been attributed to the roles played by these elements in genetic diversity and evolution of other species, as well.

Since the different mechanisms of transposition of these mobile elements result in considerable structural damage to the genome, they represent significant potential sources of apparently spontaneous mutations and an important cause of human diseases induced by their behavior.

Transposition might be an important mechanism that underlie the creation of pseudogenes in the human genome. If these genes could be induced to regain function instead of mutated genes, this might be an important genetic therapy approach to a considerable fraction of genetic diseases.

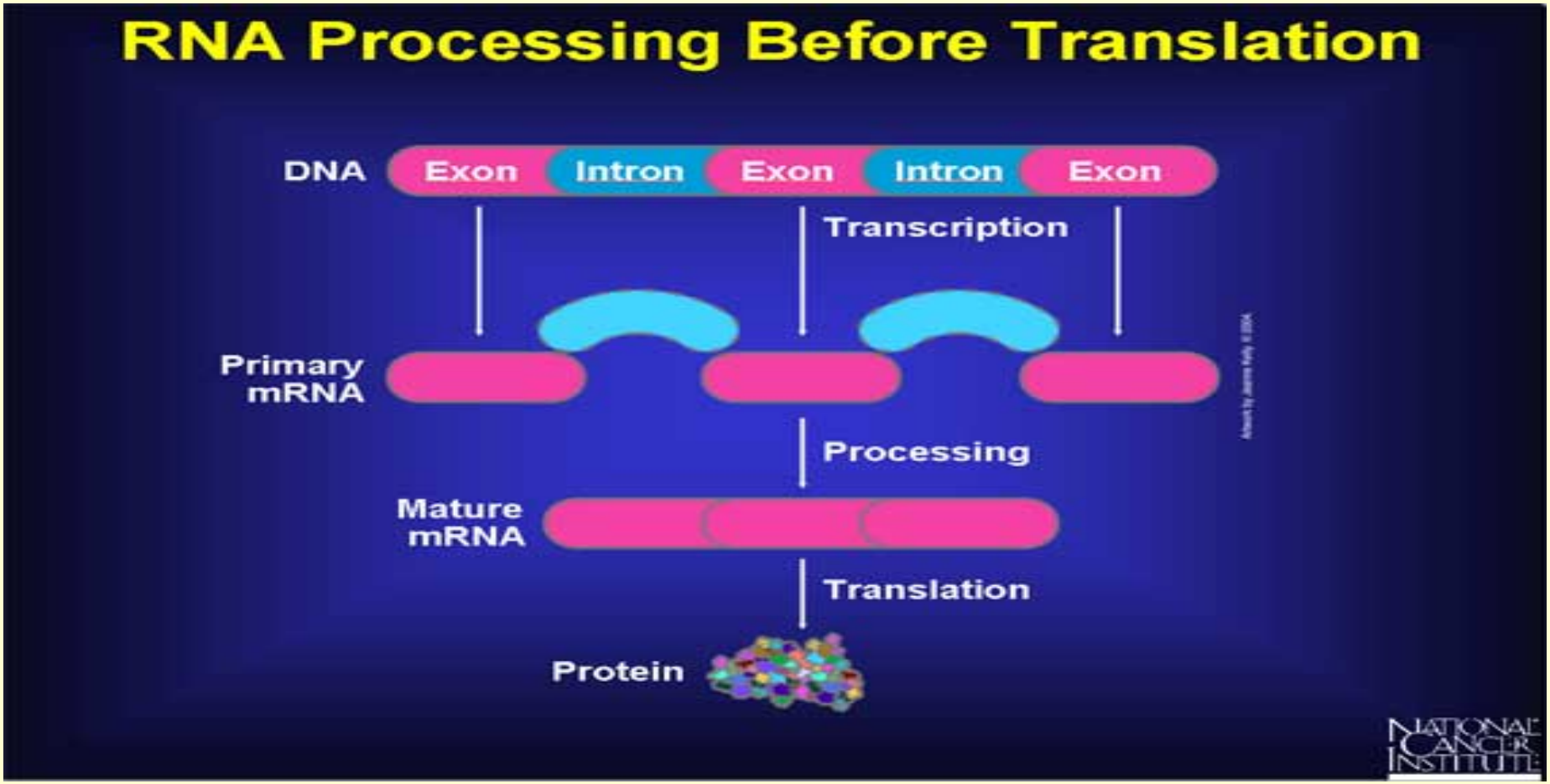
Normal Splicing Process

1. Excision Of Introns
2. Splicing Of Exons



Splice Defects-Induced Mutations

Excision of introns and splicing or joining of exons represent a critical and important post-transcriptional modification of primary mRNA ensuring proper translation of the protein.

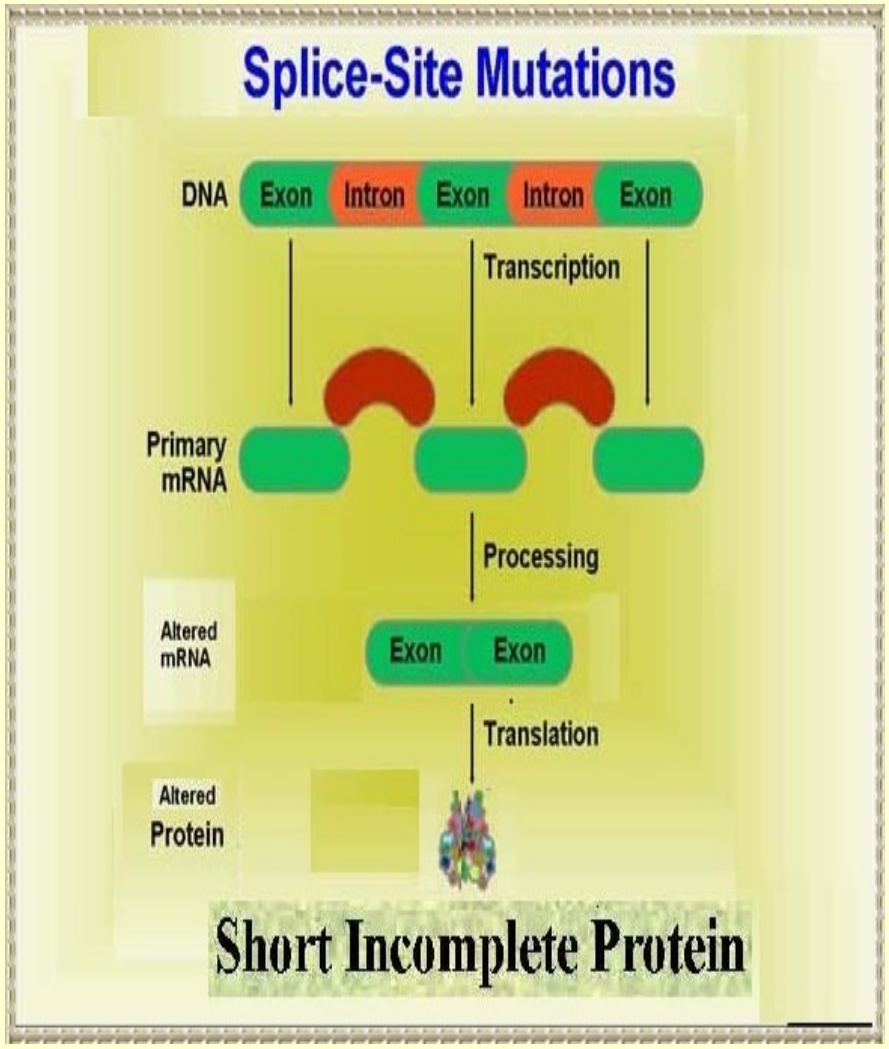


Primary mRNA splicing is mediated by a complex mechanism involving large macromolecular complex, the spliceosome, which recognizes specific sequences at intron-exon borders, including the splice sites and branch point sequences.

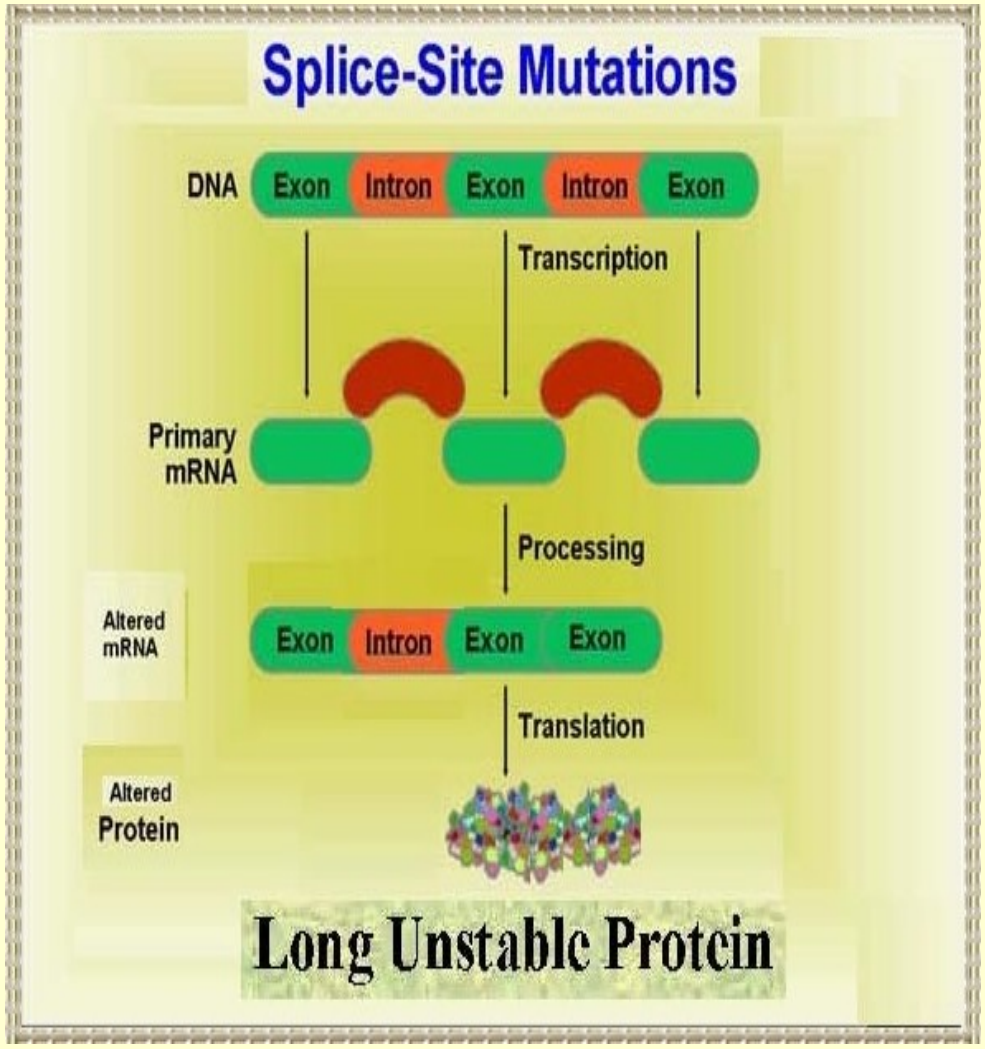
Defects in mediating proper excision-splicing processes can lead to variable deletions of exonic segments of mRNA or persistence of intronic segments of the molecule. Sometimes these deletions might comprise one or more exons leading to synthesis of shorter, defective polypeptide chain, a process called protein truncation. Defects leading to abnormal persistence of intronic segments in the final mRNA will result in synthesis of abnormally longer polypeptide chains. This can result in abnormal or defective post-translation modifications or to unstability and easy degradability of the protein, both of which are well known pathogenetic mechanisms .

Splicing Defects Mutations

Excision Of Exons

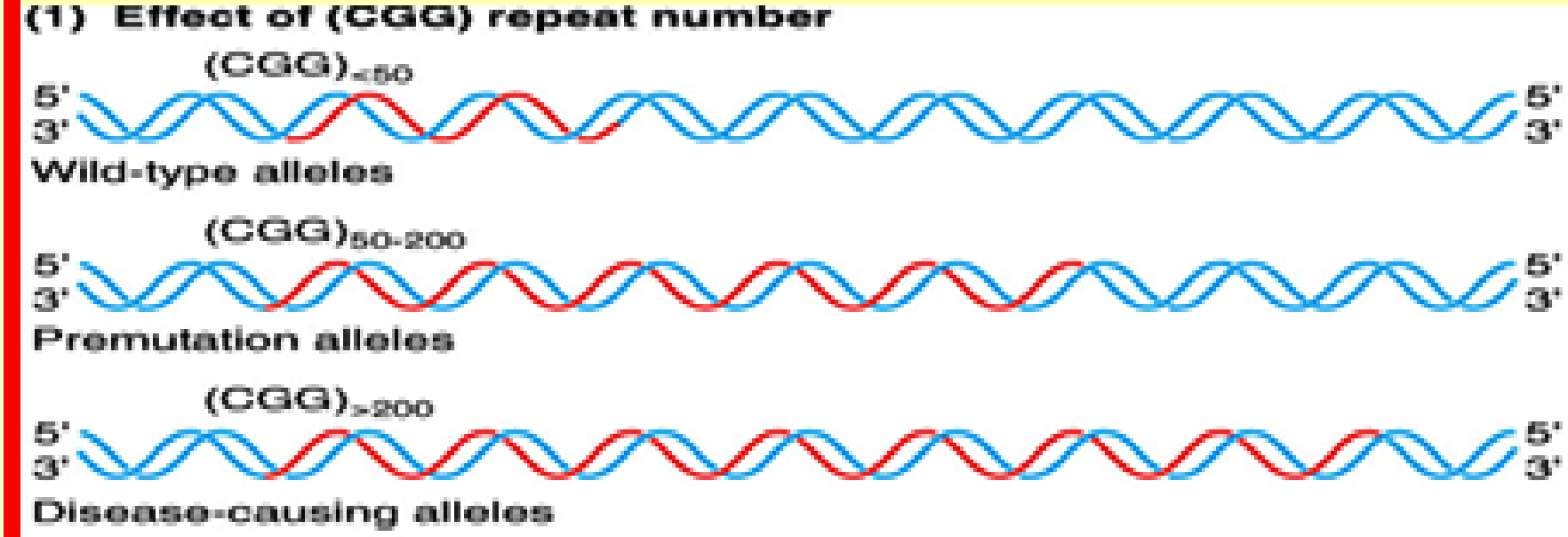


Persistence Of Introns



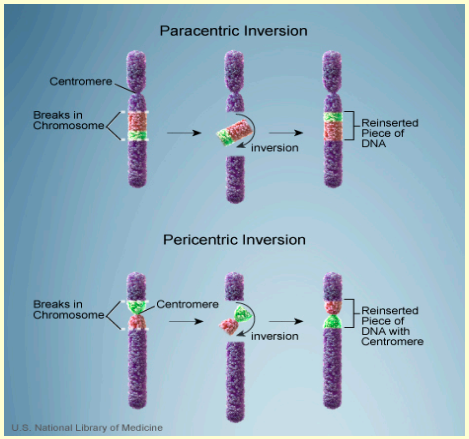
Dynamic Mutations

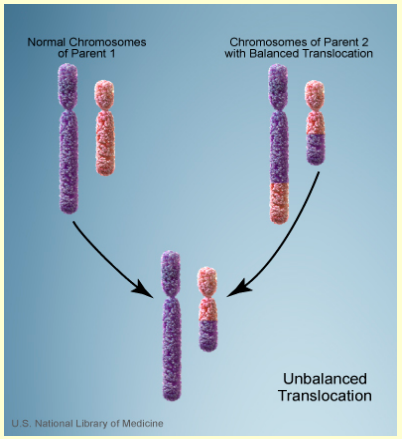
Dynamic Mutations
Fragile X Mental Retardation

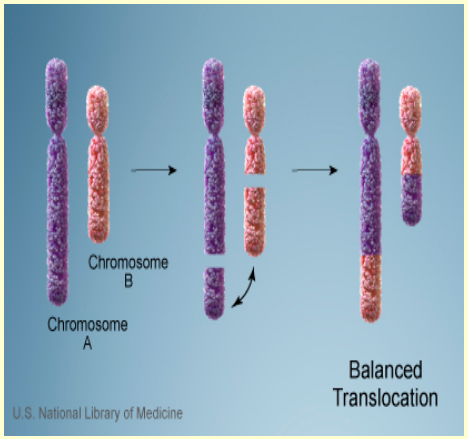


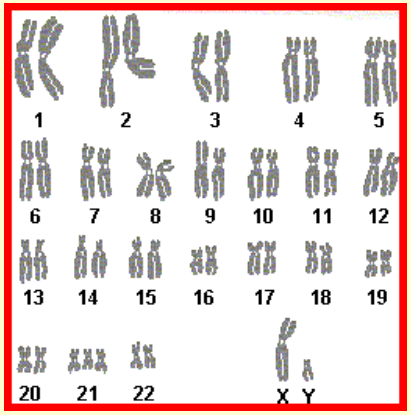
3. CHROMOSOMAL MUTATIONS

Structural Mutations	Numerical Mutations
1. Deletion	1. Trisomy (47 Chromosomes)
2. Translocation	2. Monosomy (45 Chromosomes)
3. Insertion	3. Hypodiploidy (Less than 46)
4. Ring chromosome formation	4. Hyperdiploidy (More than 46)
5. Dicentric chromosome formation	5. Triploidy (3N : 69 Chromosomes)
6. Chromosome gaps and breaks	6. Tetraploidy (4N : 92 Chromosomes)


Paracentric Inversion
Centromere
Breaks in Chromosome
inversion
Reinserted Piece of DNA
Pericentric Inversion
Breaks in Chromosome
Centromere
inversion
Reinserted Piece of DNA with Centromere
U.S. National Library of Medicine


Normal Chromosomes of Parent 1
Chromosomes of Parent 2 with Balanced Translocation
Unbalanced Translocation
U.S. National Library of Medicine


Chromosome B
Chromosome A
Balanced Translocation
U.S. National Library of Medicine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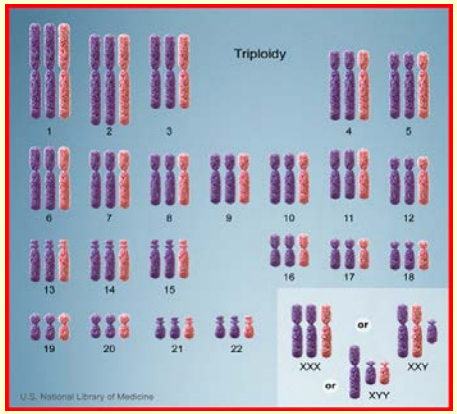
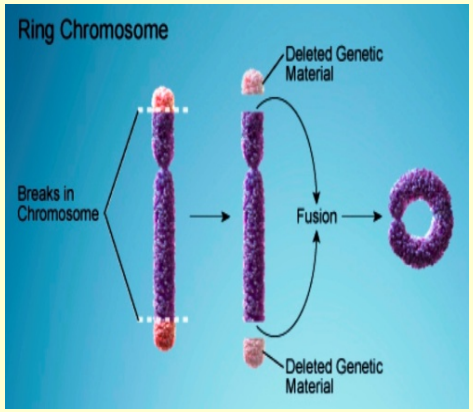
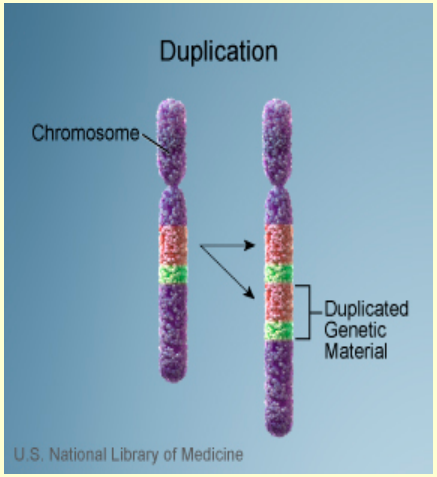
X Y


Triploidy
U.S. National Library of Medicine


Ring Chromosome
Deleted Genetic Material
Breaks in Chromosome
Fusion
Deleted Genetic Material


Duplication
Chromosome
Duplicated Genetic Material
U.S. National Library of Medicine

Chromosomal Abnormalities

A. Autosomal abnormalities

1. Autosomal Numerical Abnormalities
2. Autosomal Structural Anomalies

B. Sex chromosomal abnormalities

1. Sex Chromosomes Numerical abnormalities
2. Sex chromosomes Structural abnormalities

C. Genome abnormalities

1. Triploidy (69 chromosomes)
2. Tetraploidy (92 chromosomes)

A. Autosomal abnormalities

Autosomal Numerical Abnormalities

a. Trisomy 47 chromosomes

b. Monosomy less than 45 chromosomes: lethal, incompatible with life.

c. Hypodiploidy less than 45 chromosomes

d. Hyperdiploidy more than 47 chromosomes

Autosomal Structural Anomalies

Deletions, translocations, isochromosomes, inversions, instability, insertions, gaps, transposition, etc.

B. Sex chromosomal abnormalities

Sex chromosomes Numerical Abnormalities

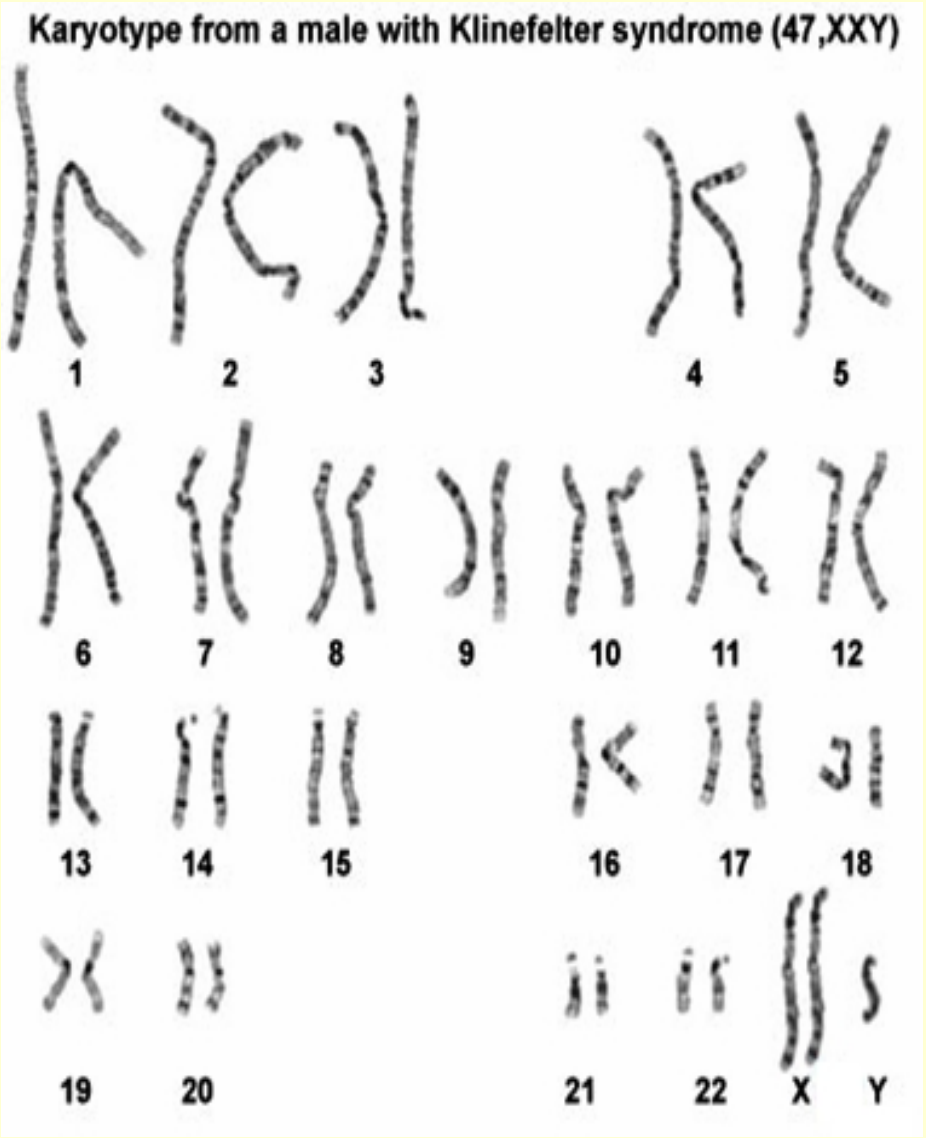
1. X-chromosome monosomy: Turner syndrome: 45,X (complete/partial monosomy).
2. 47,XXY: Klinefelter syndrome.
3. Multiple X syndromes: 48,XXX/49,XXXX syndrome
4. 47,XYY syndrome.

Sex chromosomes Structural Abnormalities

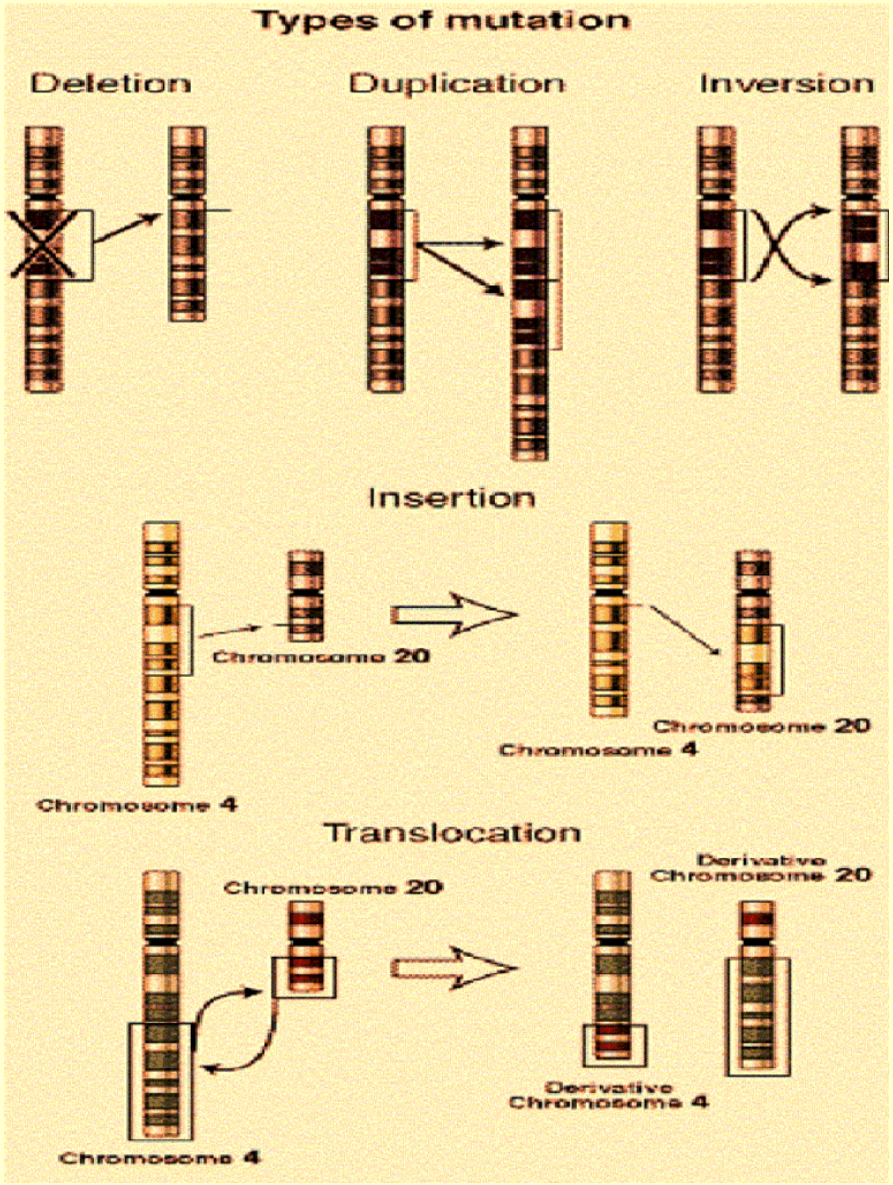
X-chromosome ring formation, partial deletion, isochromosome formaton, etc.

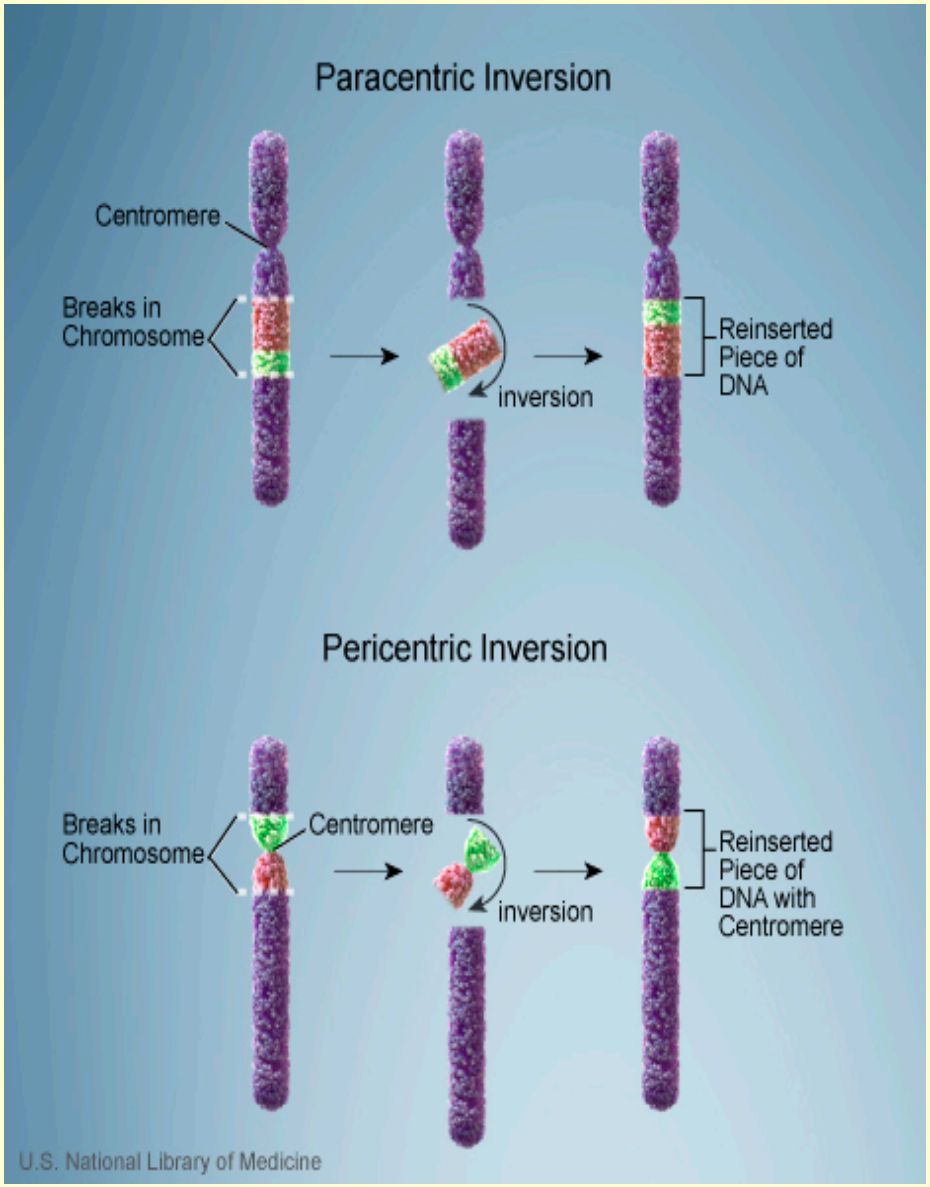
Y-chromosome deletions, microdeletions, eyc.

Numerical Chromosomal Abnormalities

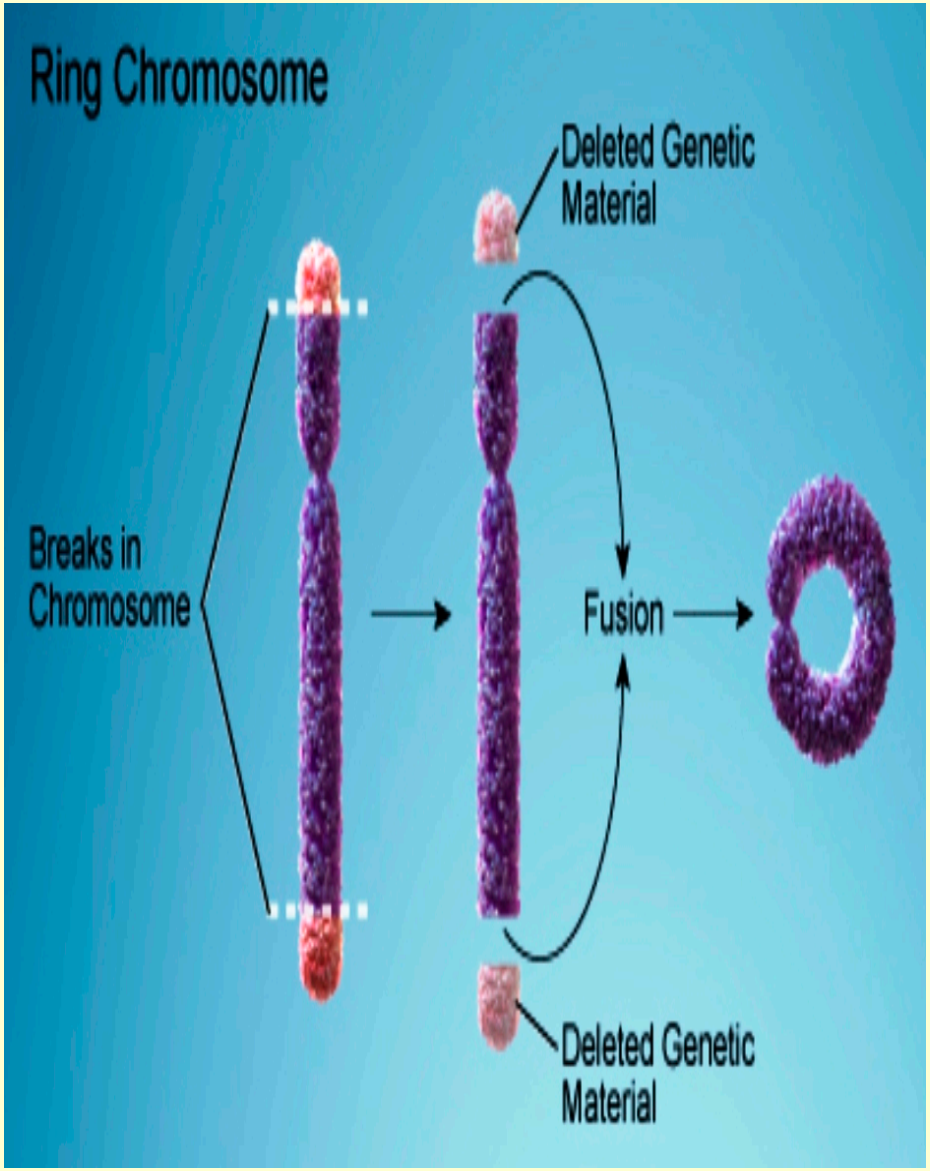


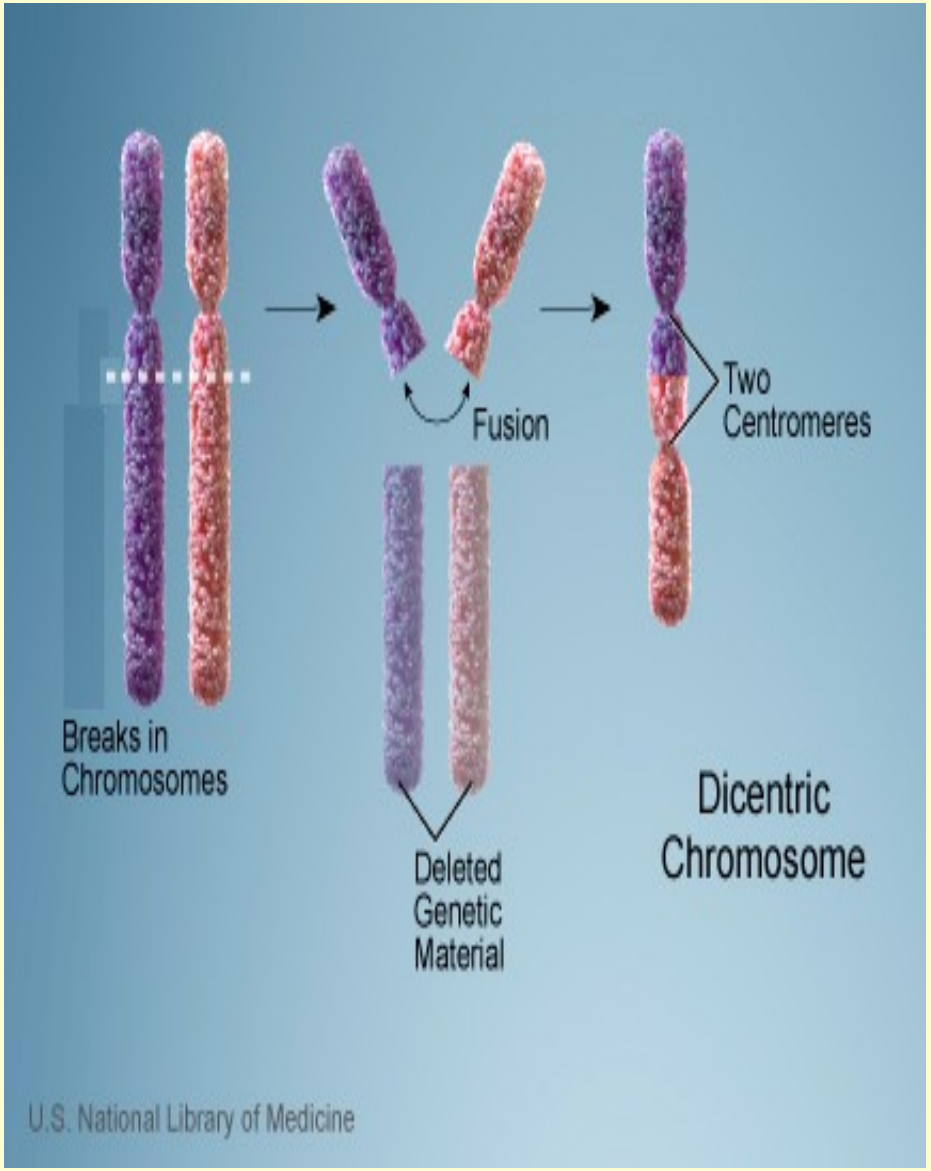
Structural Chromosomal Abnormalities



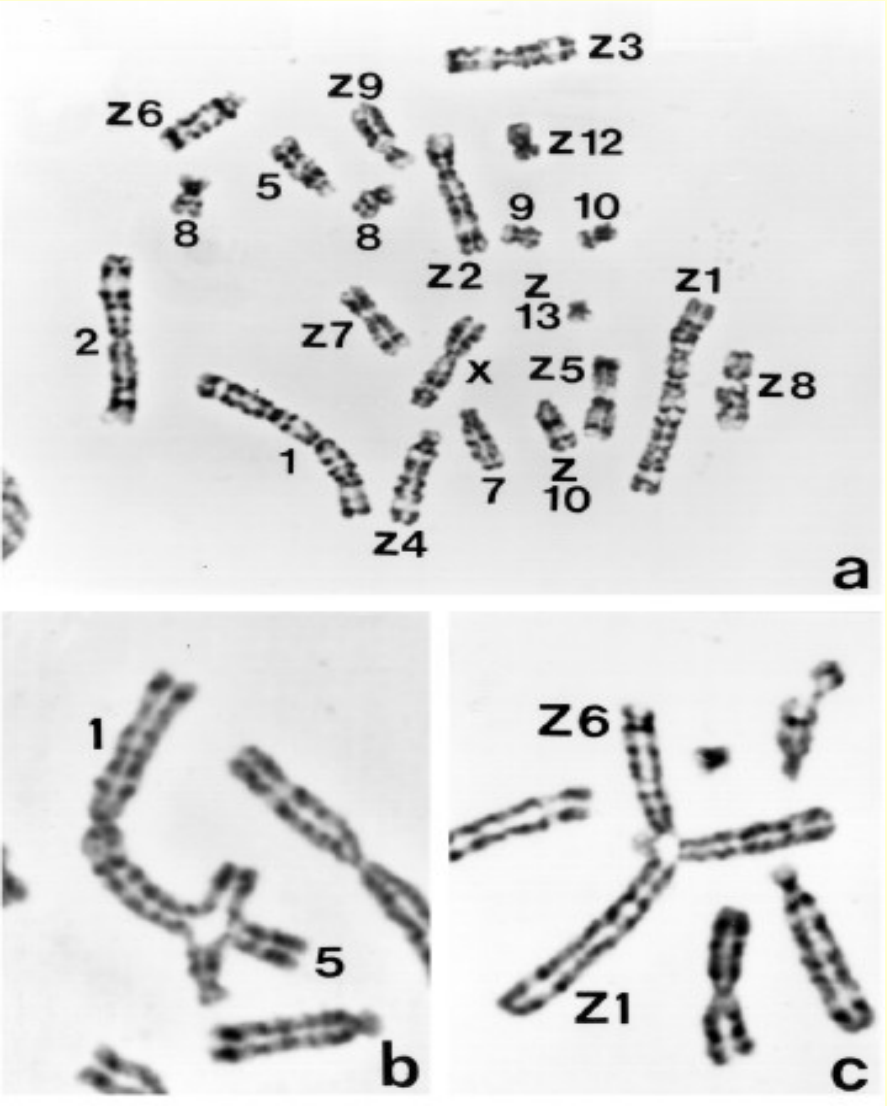


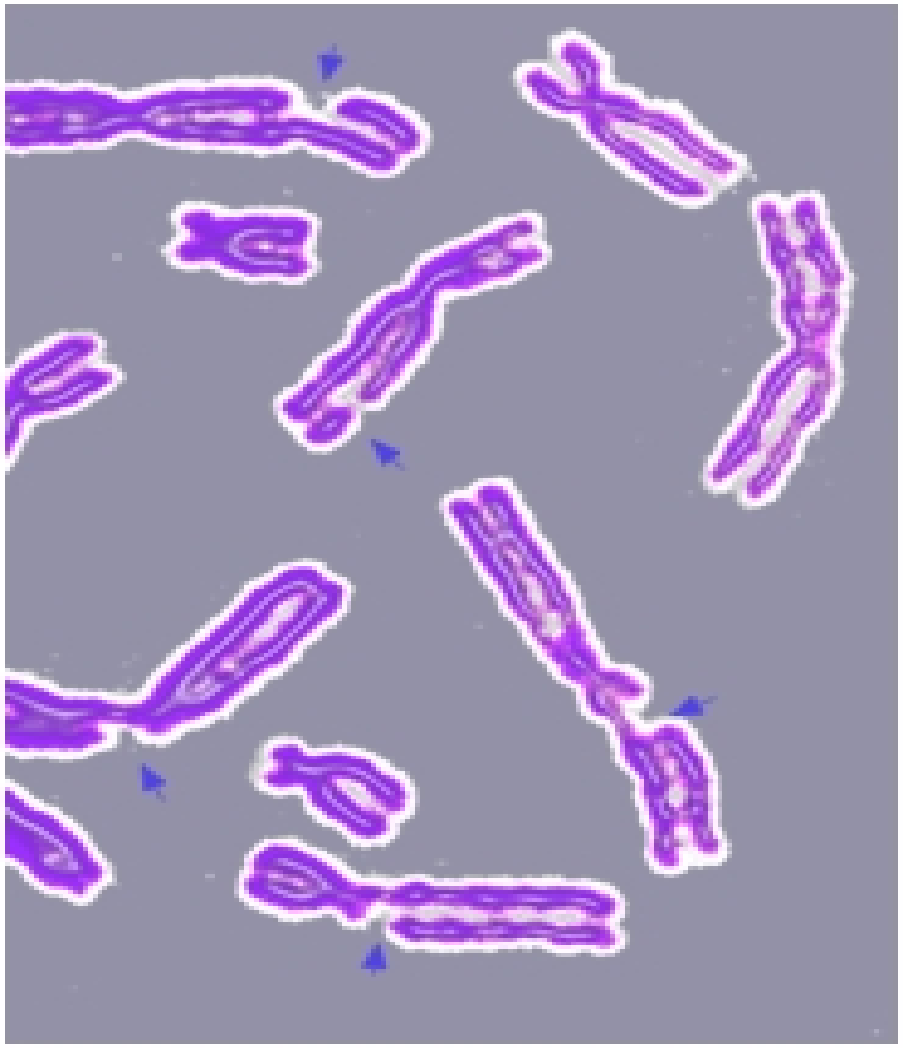
Structural Chromosomal Abnormalities





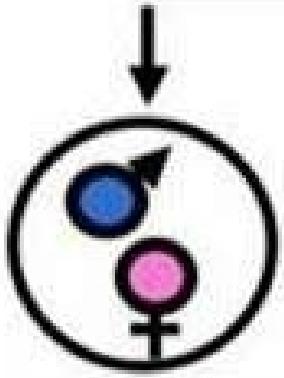
Structural Chromosomal Abnormalities
Chromosome Breakage (Gaps & Breaks)



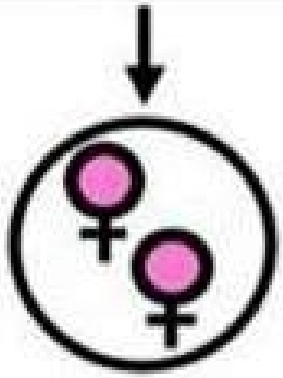


4. Genomic Mutations

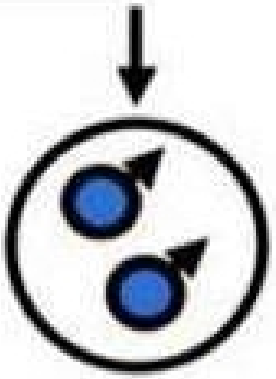
Pedigree analysis of chromosomes



Normal zygote



Gynogenetic constitution



Androgenetic constitution

PATHOGENETIC EFFECTS OF MUTATION

1. Loss–Damage-Duplication-Inactivation of gene(s)
2. Deficient / defective DNA repair
3. Loss–Acquisition–Damage of chromosome(s)
4. Deficient / defective apoptosis / selection repair
5. Deficient transcription of mRNA
6. Transcription of defective mRNA
7. Deficient / defective post-transcription mRNA repair
8. Deficient production of proteins
9. Production of defective proteins
10. Deficient / defective post-translation modifications
11. Deficient / defective post-translation protein repair
12. Deficient / defective regulation of cell growth / division
13. Deficient / defective regulation of cell differentiation
14. Deficient / defective regulation of cell migration

15. Deficient / defective regulation of cellular functions :

a. Deficient / defective transport across cell membrane or membranes of cell organelles (transport defects).

b. Deficient / defective transport across cell pores, nuclear pores or pores of cell organelles (chanellopathies).

c. Deficient / defective secretion of gene products (protein / enzyme deficiency disorders).

d. Deficient / defective excretion of metabolic waste products (storage disorders).

e. Deficient / defective regulation of intra- and inter- network reactions & interactions (signal transduction disorders).

f. Deficient / defective positioning of structural proteins (cell cytoskeleton disorders).

g. Deficient / defective regulation of intracellular trafficking.

Anti-mutation mechanisms of the human genome

The human genome develops, persists and works in a hostile micro-environment full of existing, and continuously generated, mutagens. Mutational events induced by external mutagens have widespread detrimental effects on the stability and integrity of the genome as well as on the stability and integrity of the proteome. Additionally, further and considerable damage of the structural organization and functional capabilities of both the genome and the proteome regularly occurs on continuous and progressive basis due to the

continuously generated burden of internal mutagens that result from the diverse metabolic activities of the exceedingly large number of metabolic networks of the cell.

Unless a powerful and effective protective and repair system actively participates in protecting the genome and proteome of the cell against the deleterious effects of mutations, and in efficient repair of resulting damage, maintaining the stability and integrity of both of these bio-systems that constitute the framework of life activities within the cell would have been quite impossible.

The human genome is endowed with a spectacular multifaceted strong anti-mutation system responsible for maintaining its stability and integrity, as well as preserving its identity. It acts by protecting the genome from the detrimental effects of mutation and by repairing mutation-induced damage. Obviously, the balance between the pathological effects of mutation and the ability of the anti-mutation system to counteract and to reduce the consequences of these effects represents the main factor that determines the likelihood of having and developing mutation-induced genetic disease. The human anti-mutation system comprises both innate mechanisms common to, and shared by, all individuals, e.g. degeneracy of the genetic code, and acquired aspects determined by the inherited genetic background of each human being, e.g. DNA repair system.

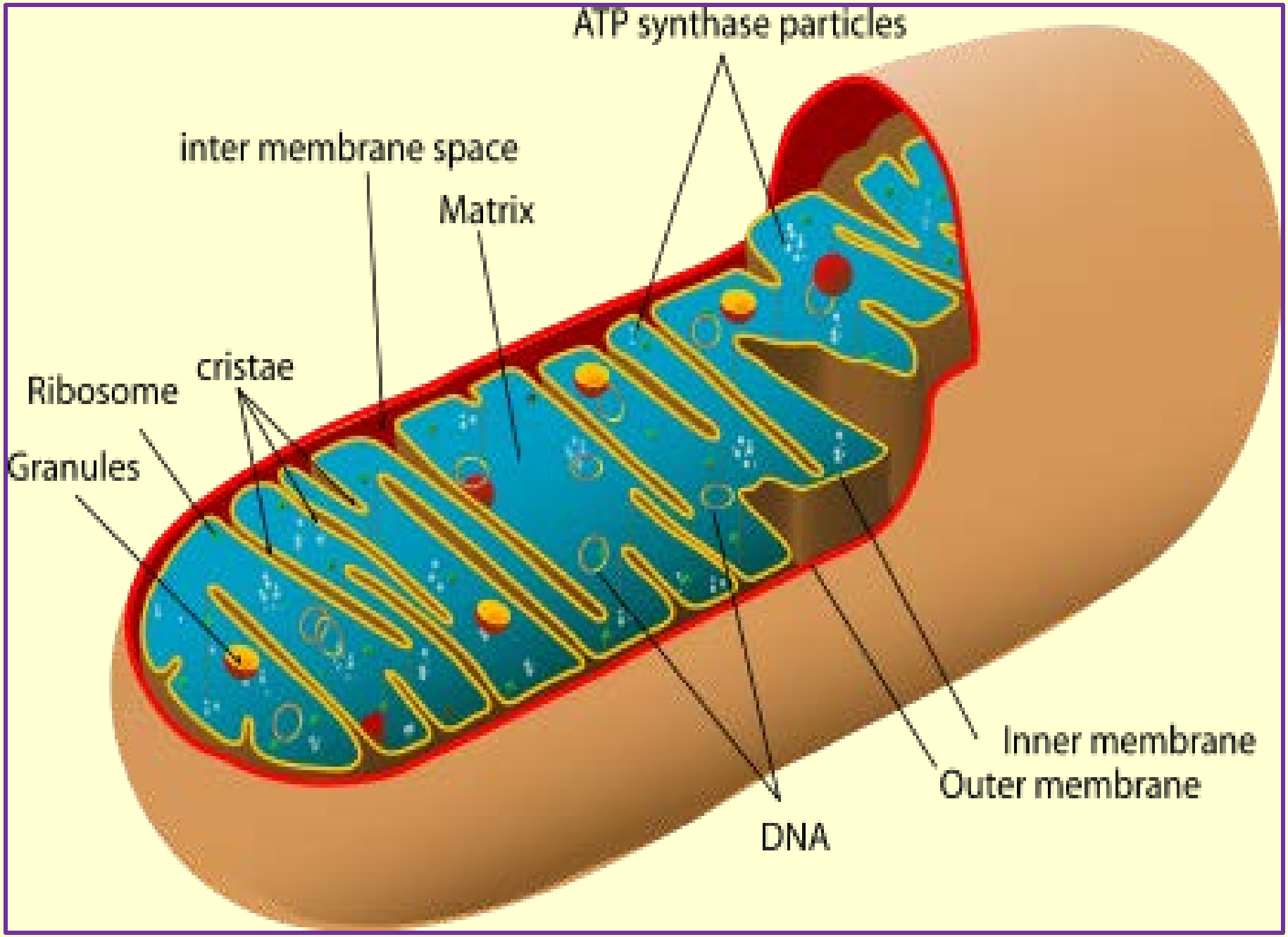
1. Structural organization of the human genome

The peculiar structural organization of the human genome represents the first innate anti-mutation mechanism in view of the presence of large interspersed portions of non-functional intragenic introns and inter-genic DNA sequences and segments that can be mutated without having appreciable deleterious functional effects. In addition to functional sequences needed for synthesis of protein and of regulatory small RNA species, the human genome has a considerable amount of repetitive DNA sequences, including both noncoding repetitive sequences and multiple copy genes and gene fragments, large number (19000-21000) of pseudogenes

a considerable sizable portion (about 1/6th of the total genome size) as pyknons, a quite large portion (nearly 40 % of the total genome size) as transposons and large numbers of multiple copies of functional genes that share the same regulatory function and whose suppression or damage by mutation can be tolerated by other genes having the same function. These peculiar structural features of the human genome allows for occurrence of mutational events in many segments of the genome without having appreciable functional defects. Even if some of these DNA sequences have important roles in genome function, their presence in multiple repetitive copies can greatly reduce, or even nullify, the consequences of mutational damage.

2. The mitochondrial genome

The presence of multiple copies, hundreds to thousands, of mitochondrial genes within the mitochondria of each cell is crucial in obviating devastating mutation-induced damage to these vital organelles in view of their role in production of ATP. This feature of mitochondrial genome allows for considerable burden of mutations to affect it before appreciable pathological consequences result. It is estimated that mutations affecting nearly 80 % of certain mitochondrial genes might occur before pathological manifestations of mitochondrial genetic diseases make their appearance due to this multiple copy feature of mtDNA.


ATP synthase particles
inter membrane space
Matrix
cristae
Ribosome
Granules
Inner membrane
Outer membrane
DNA

3. Structural features of DNA

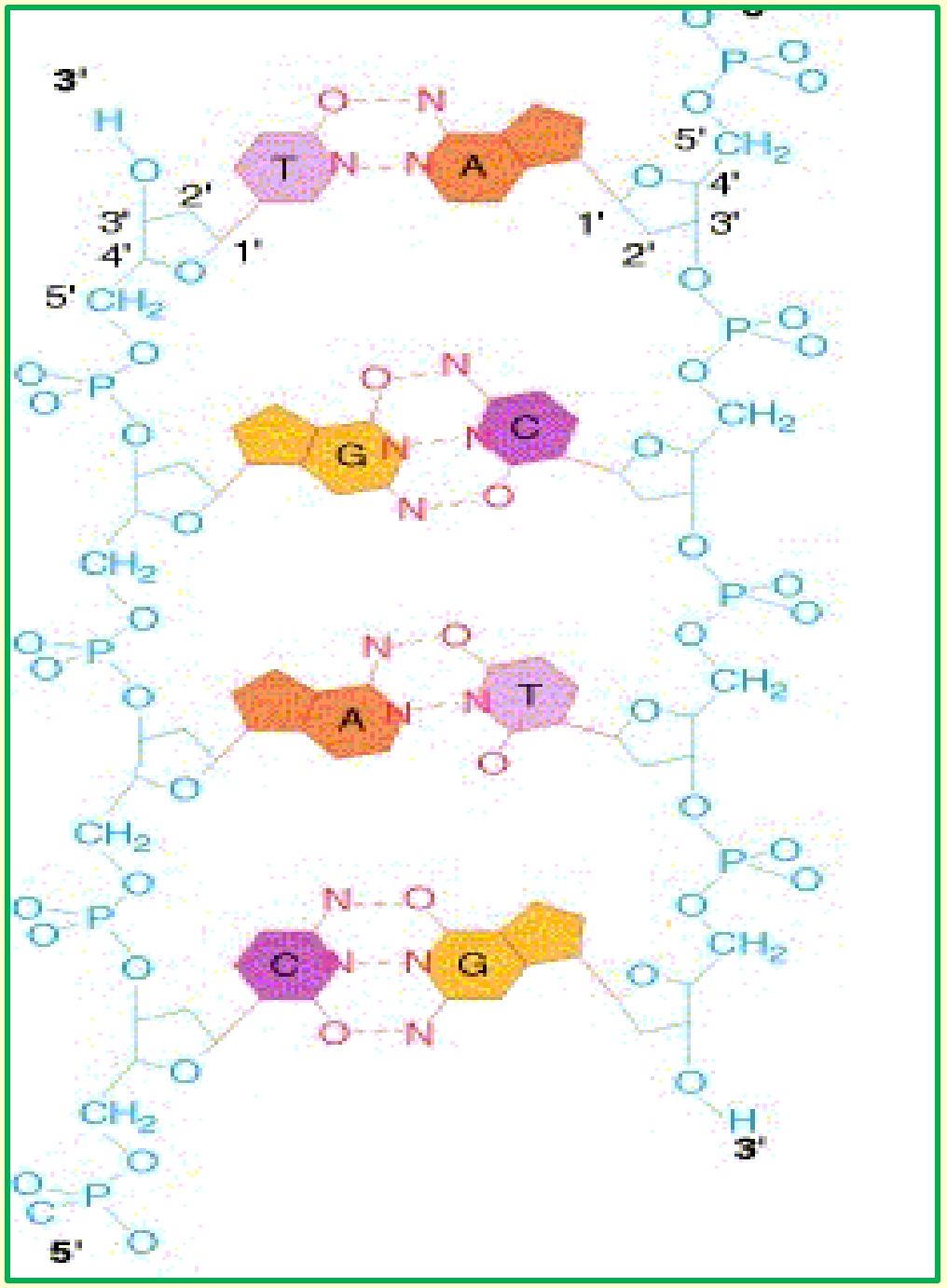
DNA exists as a double stranded structure composed of two tightly bound strands, each strand consisting of a sugar-phosphate backbone with opposing nitrogenous bases each linked by a glycosidic linkage to the sugar of its parent strand and by hydrogen bonds to the complementary base on the opposing strand. This specific structural organization of DNA serves many purposes. It stabilizes the dynamics of the molecule, permits replication and duplication of the genetic material, protects the interiorly located bases and, most important, stores a template or copy of the genetic information ready for use in case of damage of the other strand. If small or gross mutational events

affect important functional portions of the genetic material, repair mechanisms can restore the exact sequence of the damaged or lost or deleted parts through restoration mechanisms based on the complementary information of the other strand. Mutations leading to damage of corresponding segments of both strands represent a catastrophic event to the genome due to absence of the sequence database needed for the repair mechanism to define the exact base sequence of the newly synthesized segment in place of the deleted or grossly damaged segment.

Double Stranded Structure Of DNA

&

Concept Of Base Complementarity



4. Degeneracy of the genetic code

Degeneracy of the genetic code represents the third innate anti-mutation mechanism of the human genome. This feature permits the occurrence of same-sense point mutations in functional codons without changing the amino acid defined by the mutated codon. Since some amino acids, as part of a specific protein domain, play critical roles in attaining and maintaining correct protein structure and mediating proper protein function, mis-sense point mutations leading to replacement of these essential amino acids by other amino acids that can't perform the functions of the original amino acids might result in detrimental effects on the structural integrity and stability of the protein followed by deleterious consequences on its physiological function.

Hence, degeneracy of the genetic code allows for occurrence of many point mutations, the commonest type of mutational events and the commonest cause of genetic disorders, without changing the final structure of the synthesized protein, thus protecting against, and obviating, the pathological effects of these mutations.

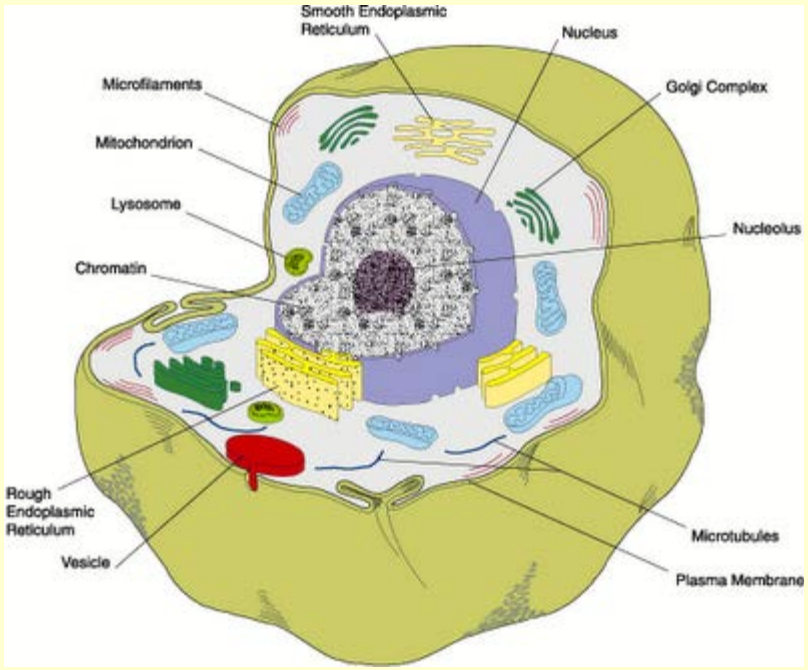
The Genetic Code

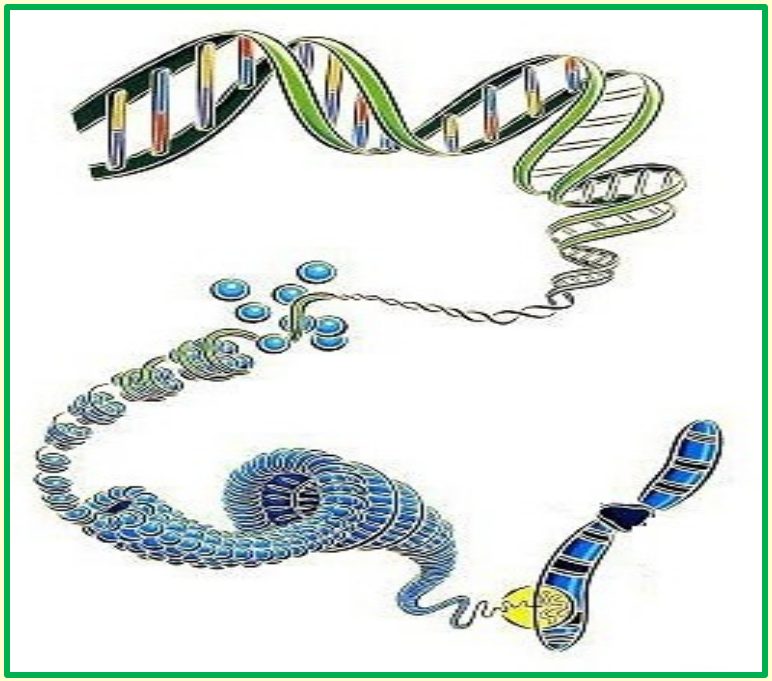
TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	STOP	TGA	STOP
TTG	Leu	TCG	Ser	TAG	STOP	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met*	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

5. Nuclear localization of DNA

The localization of DNA, deeply inside the cell nucleus, represents a fourth innate anti-mutation mechanism of the human genome because it acts as a physical barrier against many mutagens that have to overcome many obstacles of cellular defense mechanisms in order to affect the nuclear genome. These defenses include the extra-cellular environment, the cell membrane, the cytoplasmic, the cytoplasmic enzymes and phagocytic cellular organelles and the cytoplasmic and nuclear antioxidant enzyme systems. The DNA-associated or DNA-binding proteins, in addition to their essential roles in regulating transcriptional processes of most genes, also play fundamental roles in protecting the DNA from the damaging effects of many mutagens, in particular the free radicals that are generated during metabolic activities of the cell.

They act as firm physical barriers and strong biochemical buffers that effectively modify and deactivate biomolecules of many chemical mutagens or damaging factors that might harm the DNA. They mediate this protective role through many mechanisms including modulation of charge transport of oxidative agents within the DNA, limitation of DNA helix distortion and regulation of protein-dependent alterations in DNA base stacking.





6. RNA-proofreading system

The human transcriptome, being subjected to the same mutational events that can alter and damage the DNA, seems to have efficient anti-mutation mechanisms to guard against occurrence of errors during RNA transcription and to correct and repair some post-transcription defects of mRNA that can cause errors during protein translation.

Separate RNA-proofreading system seems to exist and act, probably, during transcription by relying on the sequence complementarity information of the complementary silent or non-transcribing strand of DNA, rather than of the active transcribing strand.

This behavior can be interpreted, partly, by classic principles of thermodynamics because relying on the sequence of the energetically active, transiently unstable, strand to ensure accurate transcription might result in improper defective transcription if mismatch errors occur due to, e.g. polymerase dysfunction. The risk of faulty transcription depending on the sequence complementarity information of the less energetic, more stable, non-transcribing strand is, probably, less than comparable risk expected to occur if transcription depends on the sequence complementarity information of the active strand. This assumption might, partly, offer reasonable explanation for the

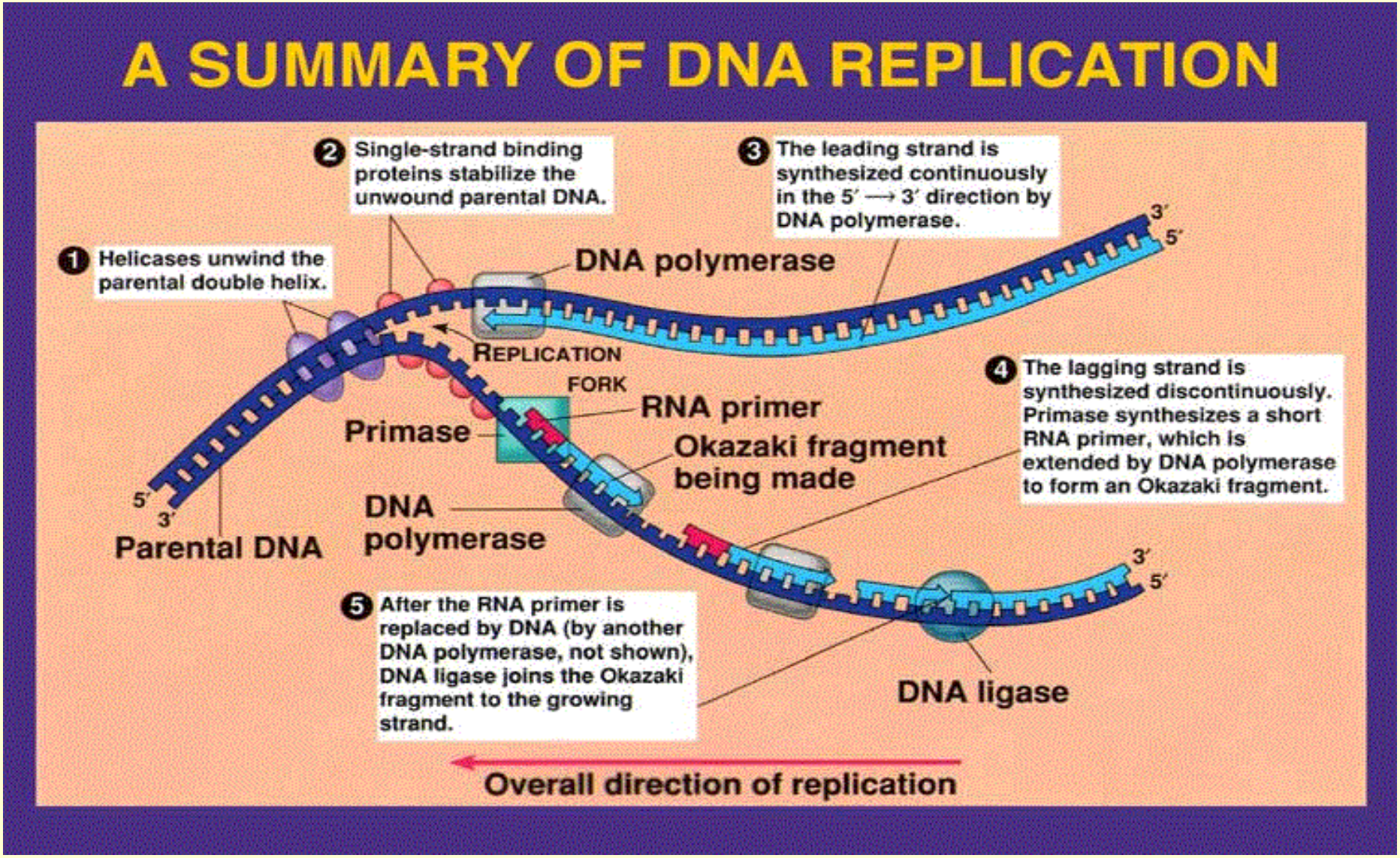
poorly understandable behavior of gene function where transcription of complementary mRNA, instead of straightforward synthesis of identical mRNA transcript, is the rule. It might also explain the seemingly needless, indirect and energy consuming processes involving transcription of complementary, rather than identical, mRNA transcripts that have to be decoded again by rRNA and tRNA in the ribosome during translation. Expenditure of energy for keeping transcriptome integrity is consistent with general rules of thermodynamics since maintaining stability of any structured system is dependent on the equilibrium between its natural tendency to degrade and external energy supply needed to keep it in a stable form.

7. Replication proofreading system

Preservation of genomic identity of the organism depends exclusively on accurate replication and synthesis of two identical copies of the genome during cell division, followed by transfer, or inheritance, of each copy to each daughter cell. In this manner, all cells descendent from a parent cell have nuclear genomes identical to that of the parent cell. The majority of spontaneous point mutations of the nuclear genome are prone to occur during cell division, mostly during DNA synthesis or the replication phase of the process.

The replication proofreading system acts in a prophylactic way to ensure accurate insertion or addition of the proper nucleotide to the newly synthesized strand of replicating DNA. This prophylactic function is fundamental to reduce the rate of inevitable replication mistakes to minimum levels that could be dealt with efficiently with the DNA repair mechanisms. In spite of the impressively fast and accurate ability of the enzymes responsible for DNA synthesis, DNA polymerases, most of them have additional proofreading ability to ensure accurate error-free DNA replication and, hence, maintaining and preserving the stability, integrity and identity of the genome during cell division, as well as during transfer of the genetic material from parents to offspring.

DNA Replication System



8. Genetic repair systems

Genetic repair systems responsible for correcting and repairing many different types of point and small mutations comprise both nuclear DNA repair system and mitochondrial DNA repair system. Genetic function and genetic repair represent two sides of the same coin. Without the persevering continuous, active and effective surveillance exerted by the genetic repair systems to detect and repair the continuously and persistently occurring mutations, maintaining stability and integrity of the genome would be an impossible task. These repair systems act via many different mechanisms according to the type, location and magnitude of damage induced in the genetic material.

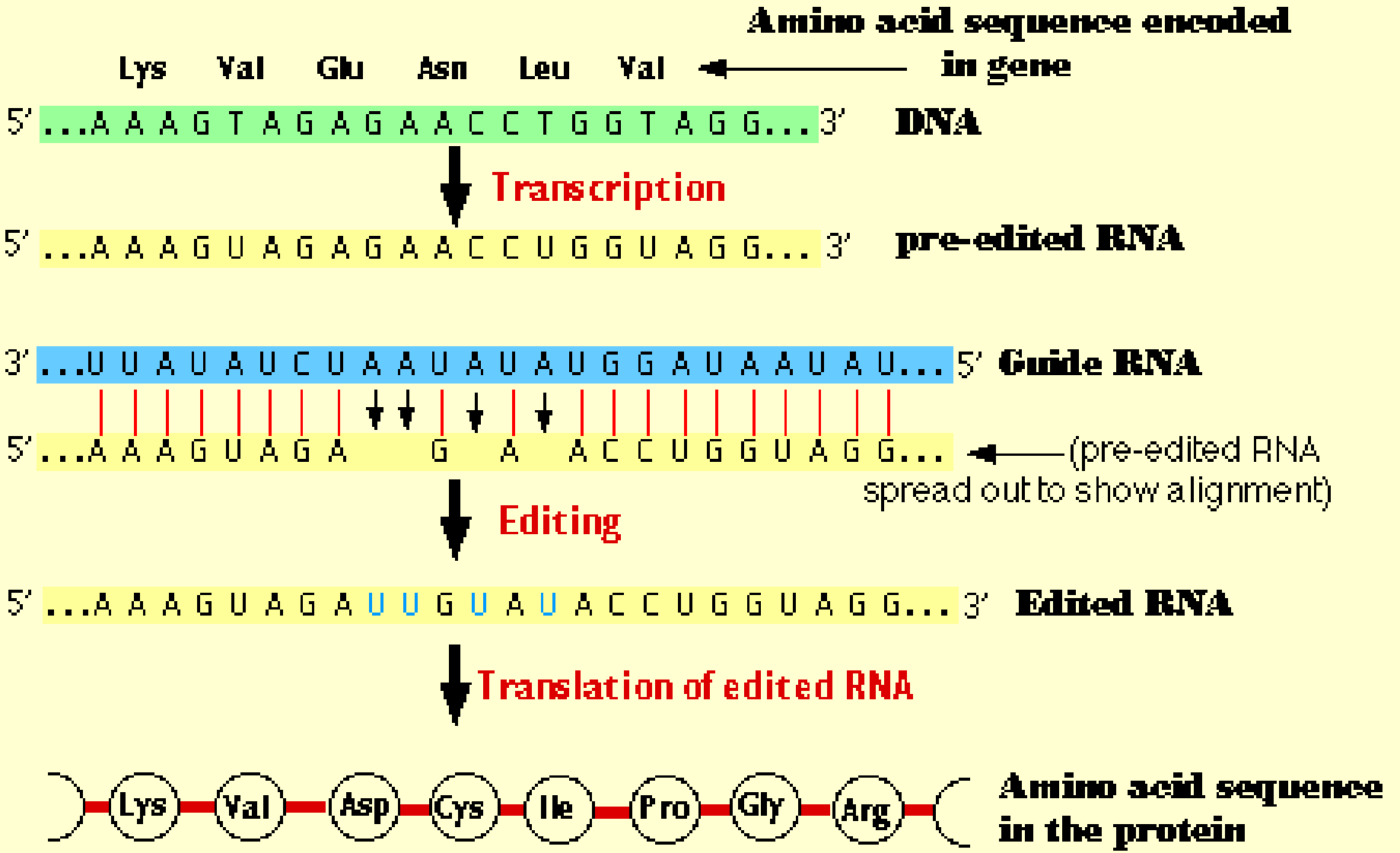
The pivotal role played by the mitochondrial genome in generating ATP, without which life can neither begin nor persist, in addition to the many other critical metabolic and regulatory functions of mitochondrial genes, requires the presence of an efficient system for repairing mtDNA mutations, mitochondrial DNA repair system. The need for mitochondrial genome repair system is further imposed on the cell in view of the high mutation rate of mitochondrial genes which lack many of the anti-mutation and protective mechanisms available to nuclear genes. Similar to the nuclear genome repair system, mitochondrial repair system includes many repair pathways and mechanisms: base excision repair, direct reversal repair, mismatch repair, and recombination repair. Nucleotide excision repair (NER) pathway, however, seems not to be working in the mitochondria.

9. RNA repair/editing system

RNA editing refers to molecular modifications of nucleotides of RNA through chemical changes in the base makeup of the molecule. Such changes appear to involve both mRNA, tRNA as well as many types of small or microRNA. RNA editing occurs in the cell nucleus and the cytosol, as well as in mitochondria and is mediated by a complex repair system comprising many species of small RNA (guide RNA) and large protein complexes known as the editosomes. The pathways and mechanisms of RNA editing include many diverse processes: nucleoside base modifications such as cytidine (C) to uridine (U) and adenosine (A) to inosine (I) deamination, as well as non-templated insertions of nucleotide.

RNA editing in mRNAs effectively alters the amino acid sequence of the encoded protein so that it differs from that predicted by the genomic DNA sequence. Though mRNA editing is used by the cell in many instances to allow for synthesis of more than one protein from the same mRNA transcript, e.g. synthesis of both apolipoprotein B-100 and apolipoprotein B-48 from the same mRNA in liver cells, it can also be used to repair missense or termination mutations of the molecule which can have deleterious effects on the synthesized protein. Specific endonucleases and ligases for double stranded species of RNA have been defined in many prokaryotes and it might be just a matter of time before defining their functional counterparts in eukaryotes and human cells.

Mechanism Of mRNA Editing



10. Protein repair systems

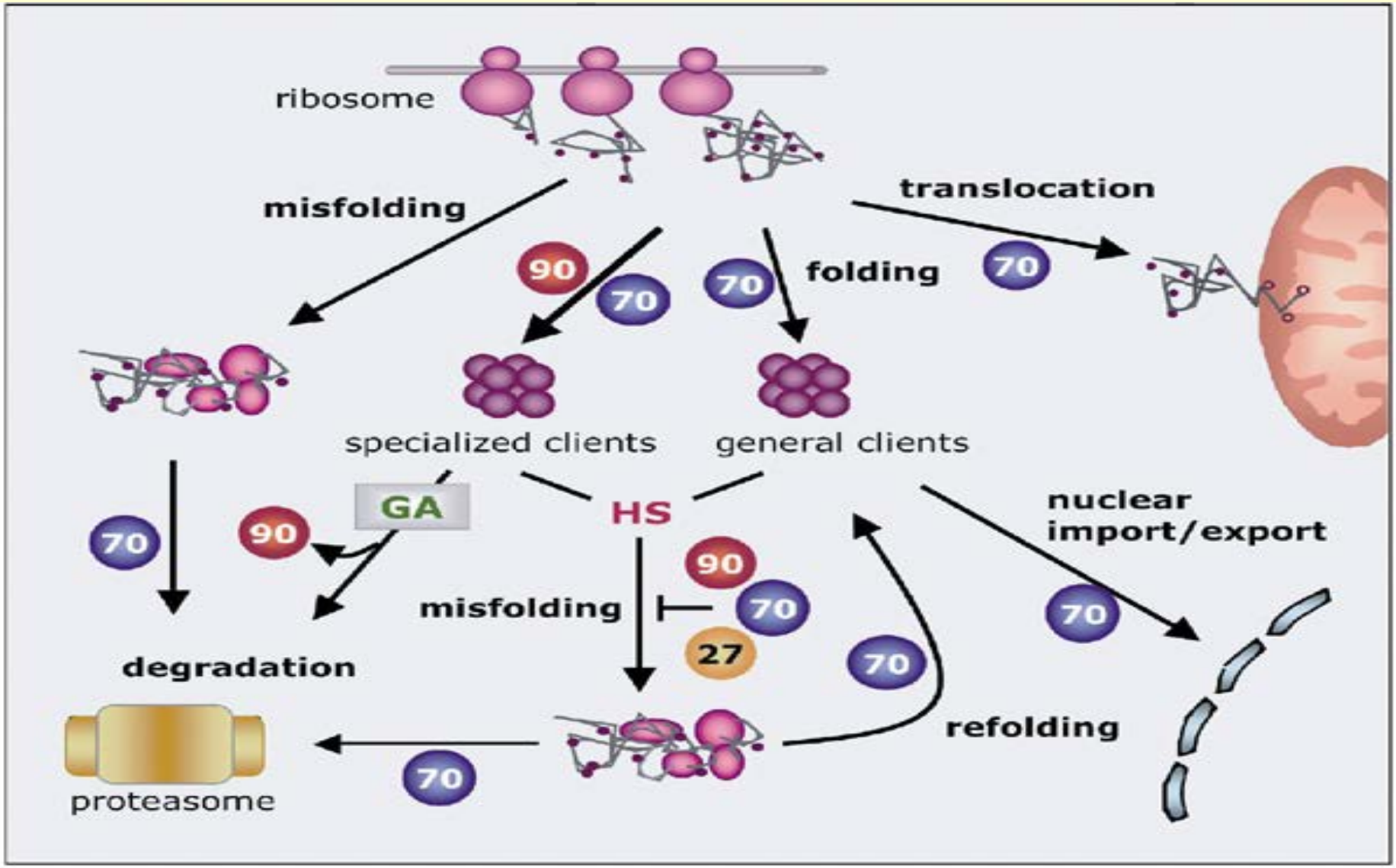
Accurate post-translation structural configuration of newly synthesized polypeptide chains is a fundamental conformational modification for most proteins to become functionally active biomolecules. The maturation from primary to quaternary protein structure involves many changes, e.g. folding and maintenance of steric and spatial relationships between the different domains of the protein. Conformational defects in proteins that might happen during these modifications can lead to formation of misfolded and/or aggregated non-functional molecules. The human genome comprises a large number of genes that code a complex system composed of large numbers of specific protein families and subfamilies known as molecular chaperones.

These proteins have many important and diverse functions in cellular activities, e.g. assisting non-covalent folding or unfolding and assembly or disassembly of macromolecular structures, including proteins.

Prevention of misfolding and/or aggregation of newly synthesized polypeptide chains, which turn them to nonfunctional biomolecules, is a major and fundamental function of molecular chaperones. Other physiological functions of chaperones include: transport across the mitochondrial membranes and the endoplasmic reticulum and assistance in protein degradation.

Molecular chaperones, probably, exert critical roles in maintaining stability and integrity of the proteome. This state of protein homeostasis, proteostasis, is a prerequisite for proper control and regulation of cellular metabolic networks by proteins and is mandatory for efficient mediation of cellular activities. Specific species of molecular chaperones, surveillance chaperones, are responsible for constant surveillance of the proteome to ensure proper protein homeostasis. Age-related decline or mutation-induced defects in proteome stability and integrity results in progressive aggregation and faulty conformational changes of proteins, both of which are associated with, and underlie, the development and pathogenesis of many genetic diseases like Alzheimer disease, Parkinson disease, prion diseases and many others.

Molecular Chaperones



11. Silencing of transposon activity during development

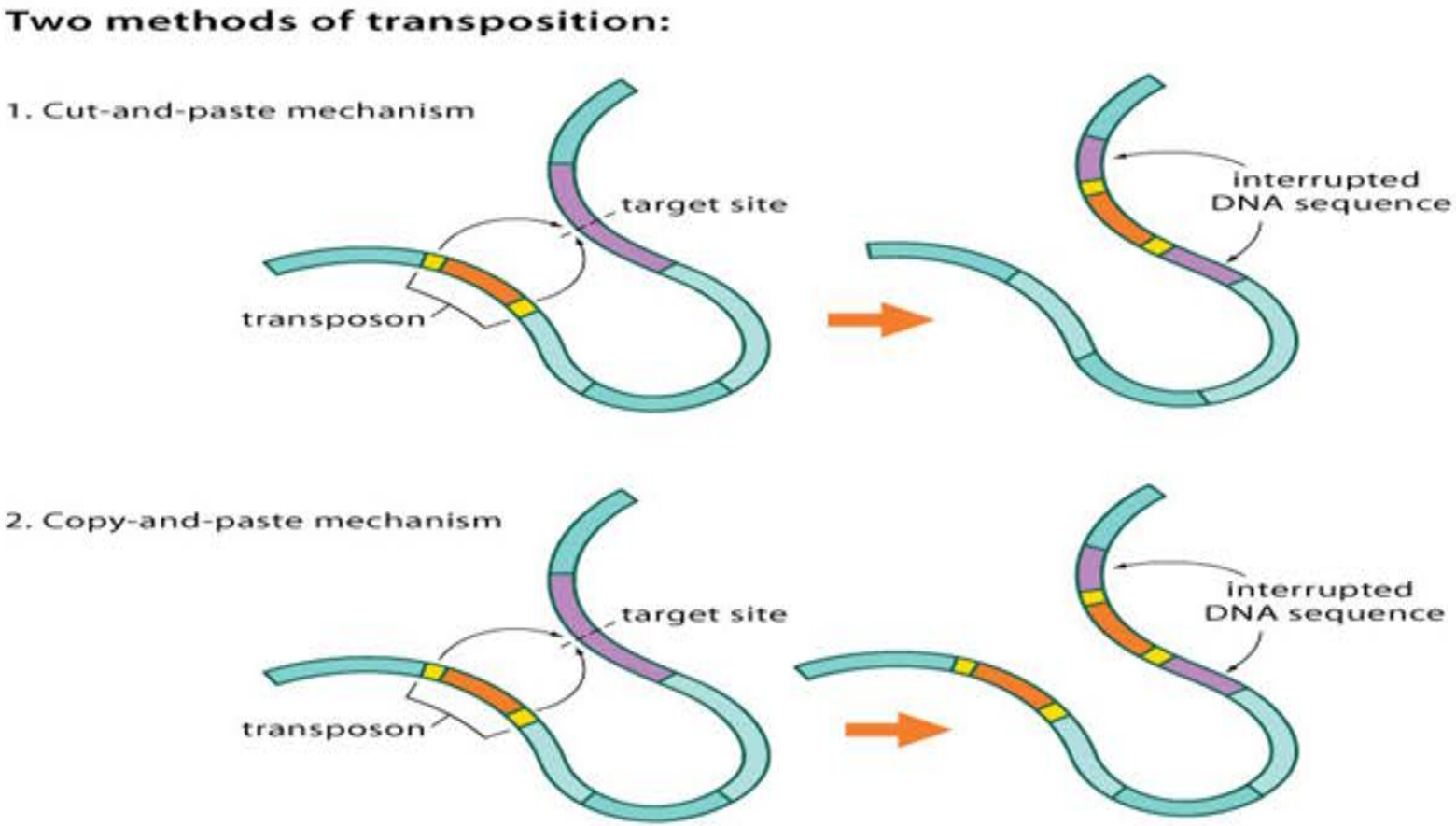
Transposons constitute a considerable portion, nearly 40 %, of the human nuclear genome. Transposon activities might have contradictory effects on the stability and integrity of the nuclear genome. They might behave in a harmful way and act as major potential causes of spontaneous mutations of the nuclear genome. They can make a copy of themselves and insert the new copy in another site, or they can detach themselves from their location and get inserted at different sites of the genome. In both conditions they result in insertional mutagenesis with consequent deleterious effects on genomic stability and genomic integrity. If they get inserted in a functional segment of the genome they lead to structural disruption and loss of function of the affected segment with resultant pathological effects.

Hence, over activity or uncontrolled activity of transposons can have detrimental and devastating effects on embryogenesis, differentiation and development, and can lead to pathogenesis of a wide variety of congenital malformations and genetic defects.

The human genome, however, has a unique control system composed of a specific subtype of small or micro RNA molecules, known as piwiRNA, or piRNA, composed of RNA-piwi protein complexes. They are thought to be involved in gene silencing, most specifically the silencing of transposons. The majority of piRNAs are antisense to transposon sequences suggesting that transposons are the main target of piRNA. In mammals, the marked activity of piRNAs in silencing of transposons and control of their activities is most important during the development of the embryo in order to reduce the rate and risk of transposon-induced mutations during this sensitive period of life.

Alternatively, transposon activity may lead to creation and construction of new genetic combinations that may have specific functions. Within this context, they would be considered as one of the genetic biological mechanisms involved in, and responsible for, evolutionary diversity of the genome and the proteome. They can also cause tangible increases in the amount of the genetic material due to recurrent synthesis and addition of multiple new copies of transposable elements to the nuclear genome.

Transposons & Transposition Mechanisms



12 Antioxidant enzyme systems

The continuous functioning of the exceedingly huge number of metabolic networks that mediate cellular activities in living cells results in continuous generation of many different types of useful as well as of harmful metabolic by-products. Oxidant free radicals constitute one of the most crucial categories of these harmful by-products in view of their ability to induce widespread damage in many cellular components including membranes, organelles and structural macromolecules like lipids and proteins. This structural damage, unless counteracted by opposing antioxidant mechanisms, results in progressive degradation of cellular constituents with consequent resultant pathophysiological alterations of cellular functions, leading ultimately to disease.

Although low concentrations of reactive oxygen species may be beneficial, or even necessary in mediating many important cellular processes, e.g. defense against invading micro-organisms and intracellular signaling pathways, nevertheless, higher concentrations of these free radicals play a causative role in the aging process as well as in pathogenesis of many human diseases, including immune deficiency, neuro-degeneration and malignancy. Oxidative damage of DNA, RNA and binding proteins by free radicals represents an important category of detrimental genetic mutations induced by endogenous chemical mutagens inevitably generated during cellular metabolic activities and other cellular functions.

Living cells have several efficient non-enzymatic and enzymatic antioxidant activities that are responsible for eliminating and/or terminating the chain reactions following generation of free radicals, as a safeguard against their damaging effects on cellular components and cellular functions.

Enzymatic antioxidant systems of the cell comprise many different types of antioxidant enzymes, notably superoxide dismutase, catalase, thioredoxin reductase, glutathione peroxidase and various other peroxidases. Efficient production of these antioxidant enzymes and proper regulation of their functions is mandatory to protect both the genome and proteome and to keep and maintain redox homeostasis of the cytoplasm which is a critical requirement for normal mediation of cellular activities.

13. Apoptosis

Apoptosis refers to programmed cell death and represents a universal biological behavior of most living cells necessary, in conjunction with other life-regulating mechanisms, for maintaining the vital balance between life and death that governs optimal life conditions of multicellular organisms. Apoptosis plays fundamental and crucial roles in normal growth and development as well as in normal differentiation and determination of the proper final structural architecture of cells, tissues and organs. Faulty timing or incorrect accomplishment of specific and selective apoptotic processes during specific life stages of the cell might result in devastating consequences on cellular functions that range from dysfunction to malformation.

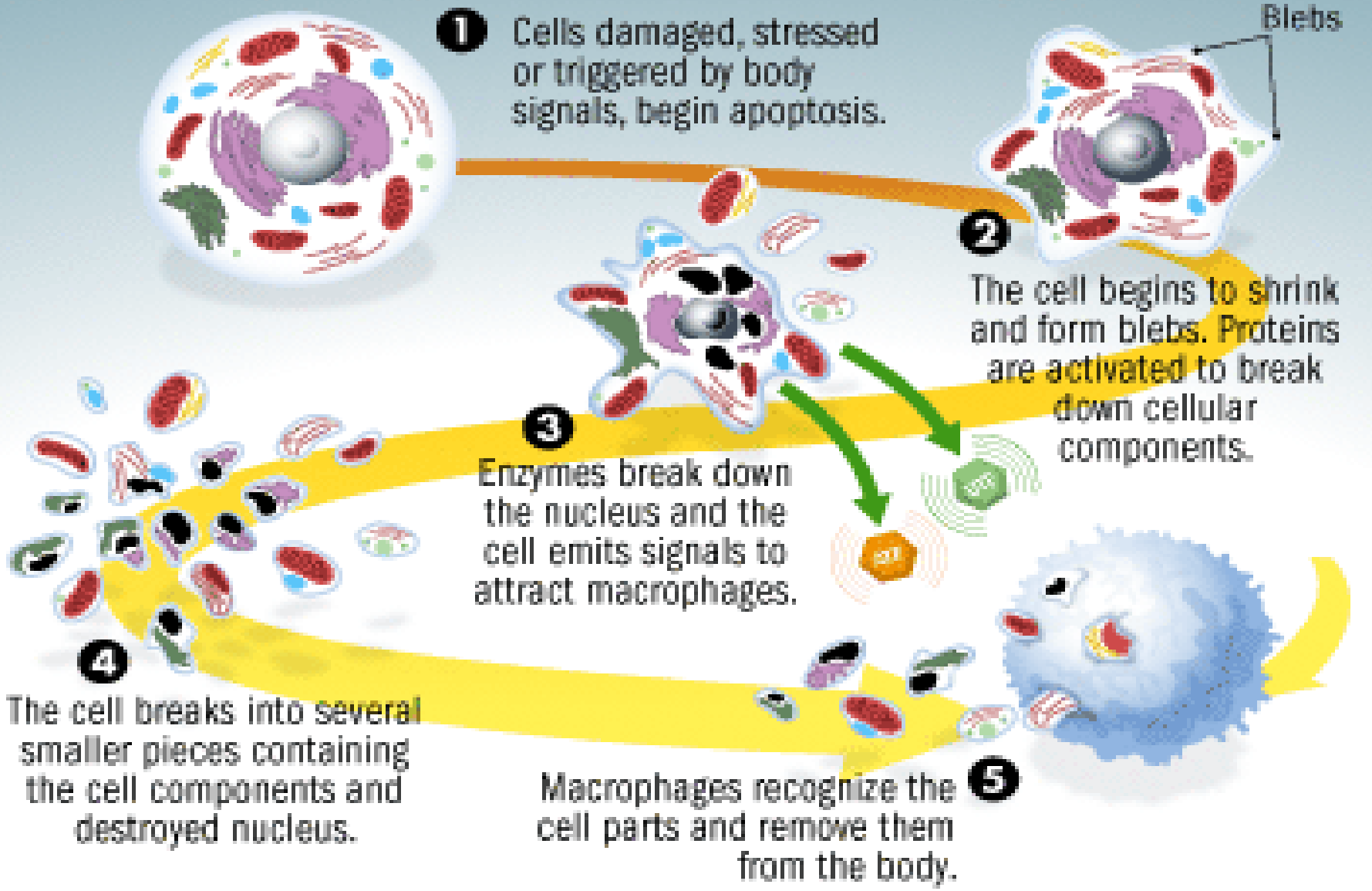
For instance, improper regulation of apoptosis during embryonic and fetal life can lead to the development of many different types of congenital malformations. In many conditions, it might ultimately culminate in pathogenesis of disease or in premature cell death.

Apoptosis plays a crucial role in maintaining genomic stability and integrity, not of individual cells, but of the organism as a whole. Induction of apoptotic mechanisms in heavily mutated or lethally mutated cells leads to death of the cell and prevents transfer of these mutations to its putative descendant daughter cells. This fundamental prophylactic anti-mutation role of apoptosis in cellular activities and life prospects of living organisms has more far-reaching effects on many important aspects related to the balance between, and the incidence of, normal and mutant genotypes within species-specific gene pools.

Additionally, apoptosis can affect in an appreciable manner genomic identity of living organisms because mutation-induced evolutionary or decadence pathways are largely dependent on the outcome of certain apoptotic mechanisms operating during certain stages of the cell cycle.

Apoptosis may be looked at as a special genomic protective pathway involving compulsory death of somatic cells overburdened with mutation. Over mutated cells are driven to their ends through specific apoptosis-mediating pathways to protect other cells from the hazardous risks of their malignant transformation. Apoptosis might, also, represent a cellular economic adaptation behavior by getting rid of mutated, diseased, energy consuming and non-functioning harmful cells. In this way, it performs important protective anti-mutation mechanism to maintain genomic integrity through executing and getting rid of heavily mutated cells, to prevent spread of their mutations, through division, to daughter generations.

Apoptosis Process



14. Melatonin

Melatonin is a hormone synthesized by the pineal gland, bone marrow cells, epithelial cells and lymphocytes. Melatonin receptors are distributed in most organs, a finding reflecting its widespread roles in regulating various physiological and psychological processes. Many in vitro and animal studies revealed that melatonin has diverse functions including effective protection of cells against radiation-induced chromosome breakage and inhibition of tumor development in animals exposed to experimental chemical carcinogenesis. Melatonin was shown to have protective effect against oxidative DNA damage by chemical inactivation of DNA-damaging agent as well as by stimulating DNA repair mechanisms.

These important anti-mutagenic and anti-clastogenic effects of melatonin can be linked with its ability to protect DNA against oxidative damage. It may exert this antioxidant action by eliminating harmful reactive oxygen radicals or by stimulating the repair processes of oxidative stress-induced damage of DNA .

Anti-mutation Mechanisms

1. Structural features of DNA & complementarity	2. DNA-associated proteins
3. Nuclear localization of DNA	4. Replication proofreading systems
5. RNA proofreading	6. Post-transcription repair
7. Degeneracy of the genetic code.	8. Transposon silencing by piwiRNA
9. Protein repair system	10. Anti-oxidant enzymes
11. Apoptosis	12. Melatonin
13. DNA repair systems	14. The mitochondrial genome

Pathogenesis Of Genetic Diseases

Genetic diseases are caused by mutations, or structural changes of the genetic material at any of its organizational levels. Mutations cause disturbances and alterations of the structure and / or function of the genetic material, leading ultimately to one or more of the following consequences :

1. Deletion, or loss, of part of a gene, one or many genes, part of a chromosome, one or more chromosomes, one or more of mitochondrial genes, or even a whole genome.
2. Duplication / Rearrangement of the genetic material.
3. Deficient / Defective transcription of mRNA.
4. Deficient / Defective post-transcriptional modifications of mRNA.
5. Deficient / Defective translation of mRNA leading to deficient / defective production of gene products.
6. Deficient / Defective post-translational modifications of proteins.

7. Deficient / Defective production of regulatory factors. These include microRNA, transcription nucleoproteins, etc.

Irrespective of the site, type, nature or magnitude of the mutational event(s) that drastically affect the genetic material, the resultant alterations in gene function(s) trigger many disturbances in one or more of the cellular metabolic regulatory networks mediated by the deficient / defective gene products, thus leading to a wide and varied spectrum of pathophysiological changes in cellular functions leading, ultimately, to development of genetic diseases.

The specific pathognomonic phenotype that characterizes each genetic disease is primarily determined by the spectrum of pathophysiological changes in affected subjects. These, in turn, are determined by the spectrum of the mutation-induced damage to the genetic material in affected patients.

Pathogenesis Of Genetic Diseases
Structural Gene Mutations

Mutation

↓

Change Of Gene Structure

↓

Promotor Mutations → Suppression & Loss Of Gene Function
Disturbance In Transcription

↓

Defective mRNA Processing / Repair

↓

Disturbed / Defective mRNA Translation

↓

Deficient Synthesis Of Protein
Synthesis Of Defective Protein
Defective Post-Translation Modifications
Defective Protein Repair
Disturbances In Metabolic Networks

↓

Protein Deficiency Disorders . Albinism
Inborn Errors Of Metabolism
Oxidative-Phosphorylation Defects

Pathogenesis Of Genetic Diseases
Regulatory Gene Mutations

Mutation

↓

Loss / Disturbance Of Gene Function

↓

Loss Of Regulation Of Cellular Processes

↓

Disturbance / Arrest Of Cell Growth

↓

Disturbance / Arrest Of Cell Replication

↓

Disturbance / Arrest Of Cell Movement

↓

Disturbance / Arrest Of Tissue-Organ Architecture

↓

Cancer . Apoptosis . Progeria
Immune Deficiency . Migration Defects
Congenital Malformations

www.archive.org/details/MedicalGenetics